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Disinfecting Contaminated Water with Natural Solar Radiation Utilizing a Disinfection Solar Reactor in a Semi-arid Region

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Abstract

The present investigation was carried out to assess the efficiency of solar energy in disinfecting contaminated drinking water in a semi-arid region. Distilled water was inoculated with coliform bacteria and exposed to up to 6 hours of ultraviolet radiation using natural solar radiation and UV lamps of 365 nm wavelengths with varying intensities. Total coliform counts were enumerated at intervals to determine percentage inactivation against time. Other factors such as water turbidity, total hardness, chlorine level, pH and temperature were also monitored. The results showed a rapid decrease in microbial counts upon exposure to solar radiation. More than 99.99% reductions were achieved after 6 hours for the bacterial communities tested under different conditions. The rate of inactivation, however, varied and was mainly affected by water turbidity and temperature during the experiments. In addition, a solar flow through reactor for irradiating contaminated water was constructed and tested. The reactor consisted mainly of a disinfection reactor, storage tanks, a submersible pump, and a light activated switching unit. Flow regulation was achieved by a light activated switching unit which controlled the submersible pump. The flow rate was adjusted so that the time it took the water to pass the reactor was sufficient to inactivate the bacteria. The reactor was tested under varying levels of turbidity using coliform bacteria as the source of water contamination and selected bacterial species. The results indicated that turbidity affected the efficiency of water disinfection, and the reactor can be a valuable tool in solar water disinfection technology, especially, for remote and rural areas. Further work is still needed before it can be concluded that solar radiation can be an effective, cost-free, technique for drinking water disinfection.

المخلص

يهدف البحث إلى تقييم كفاءة تعقيم مياه الشرب باستخدام الطاقة الشمسية في المناطق النائية. لتحقيق الهدف تم تلقيح مياه مقطرة ببكتيريا الكوليفورم وتعرضها للأشعة فوق البنفسجية لمدد تصل إلى ست ساعات باستخدام الأشعة الشمسية الطبيعية ومصابيح الأشعة فوق البنفسجية (طول موجة = 365 نانومتر وحدة مختلفة). وتم حساب العدد الكلي لبكتيريا الكوليفورم على فترات محددة وذلك لتحديد نسبة تثبيط البكتيريا حسب الزمن وتم مراقبة تأثير عوامل مثل العكارة ومستوى الكلورين والرقم الهيدروجيني ودرجة الحرارة. وبينت النتائج أن هناك تناقص سريع في عدد البكتيريا الكلي مع زيادة مدة التعرض للأشعة حيث وصلت إلى 99.99% تناقص في عدد البكتيريا بعد تعرض للأشعة مدته ست (6) ساعات. واختلف معدل التناقص في عدد البكتيريا حسب عكارة المياه ودرجة حرارتها. كذلك تم تصميم وتجربة مفاعل لتعقيم المياه مكون من مفاعل تعقيم وخزان ومضخة ووحدة تشغيل باستخدام الإنارة. وتم تحديد معدل التدفق ليتناسب مع المدة اللازمة للتعقيم ، وبعد تجربة المفاعل عند درجات مختلفة من العكارة باستخدام بكتيريا الكوليفورم وأنواع مختارة من البكتيريا، دلت النتائج على أن عكارة المياه تؤثر على كفاءة التعقيم وأن هذا المفاعل يمكن أن يكون مفيداً لتعقيم المياه وخصوصاً في الأماكن النائية. مازال هناك حاجة لمزيد من البحث قبل أن يصبح هناك مفاعل عالي الكفاءة وقليل التكاليف لتعقيم المياه.

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Keywords: Solar disinfections; Solar radiation; Solar bioreactor; UV radiation; Water disinfection;

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1. Introduction

Securing water supplies suitable for human consumption has become an increasingly difficult undertaking in many parts of the world, particularly for communities in rural areas of less developed countries. A large number of human diseases are water-borne and can cause a variety of illnesses varying from slight discomfort to death. Diseases such as cholera, typhoid fever and shigellosis for example are well-known water-borne diseases, which can cause a staggering number of deaths annually (Mc Donald and Kay, 1988). Although the use of simple technologies, such as boiling of water, can dramatically reduce the number of harmful microorganisms; in many situations the energy necessary to carry out this task may be costly or simply unavailable. In such situations, the availability of a no-cost technique may be the answer. While simple methods are available for water clarification or suspended solids' removal, including sand filtration, it is usually the remaining microbial contamination that lingers. One of the techniques that can meet the no cost requirement is the use of natural ultraviolet [UV] radiation, which are part of natural solar radiation.

Ultraviolet radiation is divided according to its' biological effects into three major components: 1- UV-C radiation, also called germicidal radiation, which occupies the range up to 280 nm, fortunately it does not reach the Earth, 2- UV-B radiation, also called sunburn radiation, which occupies the range 280-320 nm (Furusawa *et al.*, 1990) , and 3- UV-A radiation, referred to as the black light, which occupies the range 320-400 nm (Acra *et al.*, 1990). There are conflicting reports on the wavelengths of the spectrum of radiant energy responsible for microbial inactivation (Aas *et al.*, 1996; Burkhardt III *et al.*, 2000; Kapuscinski and Mitchell, 1983; Sinton *et al.*, 1994). The damaging effects of UV radiation appears to be largely due to their formation of pyrimidine dimers, thus interfering with, or cleaving of, the nitrogen base sequences of the DNA strands in microbial cells (Muller-Niklas *et al.*, 1995). Other mechanisms for UV damage of cellular membranes (Moss and Smith, 1981) or inhibition of tRNA synthesis (Kubitschek and Doyle, 1981) have also been reported.

Studies on the use of solar radiation as a means of water disinfection started early in the eighties (Acra *et al.*, 1980). These investigations were developed to determine if the technique is effective in disinfecting small volumes of water for use in the preparation of oral rehydration solutions, used in the treatment of diarrhea. Later, Odeyemi *et al.* (1988) examined the germicidal effects of solar radiation on water samples, using transparent containers and reported total removal of three orders of magnitude [$3\log_{10}$] coliforms within 3 hours exposures. Inactivation of fecal bacteria in drinking water by solar heating have also been reported (Joyce *et al.*, 1996). Within 7 hours no viable *Escherichia coli* organisms were detected at either the end of their experiment or a further 12 hour later. Numerous other investigations have also been reported on the use of UV irradiation as an alternative technique for the disinfection of partially treated

wastewater (Acher *et al.*, 1994; Burkhardt III *et al.*, 2000; Dizer *et al.*, 1993; Job *et al.*, 1995; Meng and Gerba, 1996; Sawai *et al.*, 1993) or contaminated fresh and sea waters (Carnimeo *et al.*, 1994; Christoulas and Andreadakis, 1995; Elkarmi, 1998; Lund and Hongve, 1994; Mc Guigan *et al.*, 1998). The biosimetric measurement of the influence of reflection on the reduction equivalent fluence was investigated by Sommer *et al.* (1996) in flow through reactors.

Although the efficacy of using solar radiation for the disinfection of water has been studied by a number of investigators, very few have designed and tested pilot reactors that utilize this method to come up with a simple and low cost system that can be used in places where contaminated drinking water is a health hazard. A model was designed and tested in Thailand, using copper, glass and galvanized steel as tubes for the disinfection process (Kootatop *et al.*, 1988). The results obtained from their design showed that the glass tubes provided some disinfection capabilities. Acra *et al.* (1990) designed prototype solar units using serpentine-shaped transparent tube or two Pyrex glass containers and a Pyrex glass helix. The use of an exposure vessel of 1x 2 meters with baffles for directing water flow was also used to design a reactor for water disinfection (Solarte and Dierolf, 1995). They reported that the reactor in which water is heated and exposed to solar radiation produced significant reductions in fecal coliform and *Streptococcus* concentrations under all operating conditions. The Austrian National Standard (Anonymous, 2001) provides biosimetry as the standard method for type testing of disinfection plants for drinking water (Cabaj *et al.*, 1996; Sommer *et al.*, 1997; Sommer *et al.*, 1999).

In this paper we investigated the use of solar radiation in the disinfection of contaminated drinking water in a semi-arid region and the effects of factors such as turbidity and temperature on the efficiency of UV radiation in the UV- range in the inactivation of contaminated drinking water. In addition, we explored the design and testing of a solar drinking water disinfection reactor which utilize UV radiation as the method of bacterial inactivation taking into consideration the factors that could influence the inactivation process.

2. Materials and Methods

2.1. Phase I: Laboratory Scale "Static System"

Test tubes [25 X 200 mm] with screw caps, borosilicate beakers 2 liters in volume for the preparation of contaminated water, water bath [GFL 1002-1013 series, Germany] and UV lamps and stands with wavelength of 365 nm [Cole- Parmer 9815 series, USA] were used to carry out the tests. The water samples used in the tests were prepared by contaminating distilled water with coliform bacteria. The total coliform count [TCC] was measured [initial count] using the Standard Methods-Microbiological Examination, multiple tube fermentation technique which is based on carrying out the presumptive phase, confirmed phase and completed phase (American Public Health Association, 1998). These test samples were

either subjected to the UV lamps with UV-365 nm irradiances of 4, 7, and 11 W/m² at a distance of 10 cm (according to the manufacturers information), or were subjected to direct sunlight. To test the effects of temperature, the tests were carried out at temperatures of 25, 30, 35, 40 and 45 °C using a water bath to vary the temperature. The test tubes were tilted by 45° angle so that the water in the water bath covered one side of the tubes and the upper sides were subjected to UV. Samples were periodically taken every one hour or half an hour and the TCC was measured using the Standard Methods-Microbiological Examination (American Public Health Association,1998). Furthermore, turbidity, pH, total hardness, and chlorine level were measured at the start of the experiment using the Standard Methods (American Public Health Association ,1998). Tests were carried out at the Hashemite University located in Zarqa city, Jordan (altitude of 600 meters, 31° N and 35° E). At all tests control samples were prepared the same way the test samples were prepared but placed in complete darkness or covered by foil paper.

2.2. Phase II: Pilot Scale “Flow Through System”

2.2.1. Bacterial species

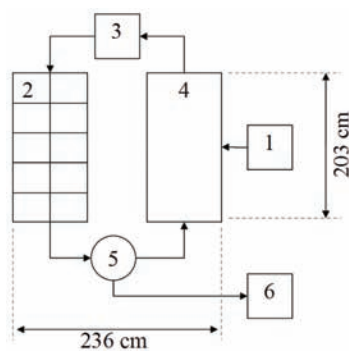
Distilled water was contaminated with coliform bacteria as described above, and total coliform count (initial count) was also measured (American Public Health Association ,1998). Furthermore, isolated bacterial species of *Staphylococcus* spp., *Salmonella* spp., *Streptococcus* spp., *Pseudomonas* spp. and *Escherichia coli* were used to examine the efficiency of the reactor in inactivating the bacteria. These wild bacterial species were kindly supplied by Jordan University Hospital-Amman, Jordan. These bacterial species were inoculated into a nutrient broth, washed twice with normal physiological saline and finally suspended in 5 ml of nutrient broth before being added to the holding tank of the reactor.

2.2.2. Reactor design

The reactor consisted of an isolated large tank (500 L) connected by PVC (1.25 cm) tubing to a smaller (125 L) tank. The water level in the small tank was controlled by a float valve. A submersible pump (Aqua Clear Power Head 200 - USA) placed in the water holding tank pumped the water to the reactor made of transparent polyethylene terephthalate (PET). The reactor was held by a metal frame similar to that of flat solar water heaters. The frame was tilted by 45 degrees facing south to ensure maximum radiation interception. The reactor was connected to another small tank (125 L) used for the storage of disinfected water. The pump was controlled by a light activated switching unit that utilizes a photovoltaic cell to switch the pump on when light is available dictated by the output voltage of the photovoltaic cell (two hours after sunrise) and off when light is not available (one hour before sunset).

It is worth mentioning that the system was modified to eliminate the need for a pump and to increase the temperature of the water by installing a flat solar water heater which can circulate the water in the reactor as well, and a heat exchanger to regulate the temperature of the

water coming out of the reactor and reduce the time needed to heat the water by the solar heater, as shown schematically in figure 1. The solar water heater depends on convective circulation established from solar heating to move the water from one tank to the other. The heat exchanger during the operation of the system will aid in increasing the temperature of the water going into the solar disinfection reactor in order to decrease the time needed to raise the temperature of the water. The system was also equipped with a thermal one-way valve to regulate the flow of water between the solar heater and the reactor. In other words, the one-way valve opens when the temperature of the water reaches the desired temperature and thus starts the whole process of disinfection (in the morning) and closes when the temperature of the water decreases below the limit (close to sunset). This modified system eliminated the need for a pump to circulate the water, a system to switch the pump (or system) on or off and increased the temperature of the water to the required level.



- 1 Water supply tank;
- 2 Solar water disinfection reactor;
- 3 Temperature regulation valve;
- 4 Solar heater;
- 5 Heat exchanger;
- 6 Storage tank;

Fig. 1. Schematic Diagram of the Modified Solar Water Disinfection Reactor

After irradiation of the bacterial suspension in the reactor, aliquots were taken in hourly intervals, and microbiological examination was performed according to standard methods (American Public Health Association, 1998).

3. Results

3.1. Laboratory Scale “Static System”

The effect of turbidity on the inactivation of coliform bacteria by UV-365 nm radiation obtained in the laboratory static system is shown in figure 2. The efficiency is calculated as the semi-log of inactivation of bacterial after 6 hours of continuous exposure to natural UV.

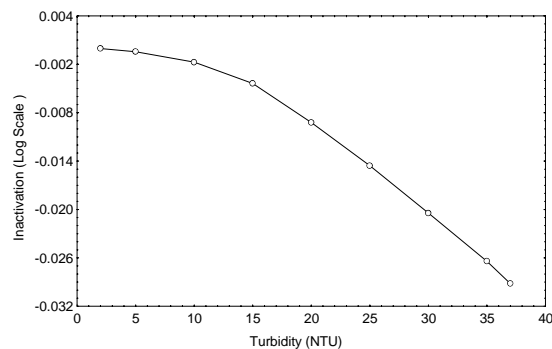


Figure 2. Effect of Turbidity on the Inactivation of Coliform Bacteria Obtained in the Laboratory Static System.

Tables (1-4) show the total coliform counts and reduction percentages after exposure to natural solar radiation under different experimental conditions of various turbidity levels measured as NTU (Nephelometric Turbidity Units), hardness and pH values in the static system. UV irradiances during tests ranged from 4-27 W/m².

Table 1. Total coliform counts and inactivation percentages at a 37 NTU turbidity water in a static system.

Exposure time (hours)	Total Coliform counts/ 100 ml	Inactivation %
0.0	1.1 x 10 ⁷	0.0
1.0	3.0 x 10 ⁶	72.7272
2.0	1.3 x 10 ⁶	88.1818
3.0	9.0 x 10 ⁴	99.1818
4.0	2.4 x 10 ⁴	99.7818
5.0	5.0 x 10 ³	99.9545
6.0	8.0 x 10 ²	99.9927
Control	5.0 x 10 ⁷	0.0

Experimental conditions: Water turbidity = 37 NTU, hardness = 273 CaCO₃ mg/L and pH = 7.1. Total time in full sun = 7 hours.

Table 2. Total coliform counts and inactivation percentages at a 27 NTU turbidity water in a static system.

Exposure time (hours)	Total Coliform counts/ 100 ml	Inactivation %
0.0	1.7 x 10 ⁷	0.0
1.0	8.0 x 10 ⁶	52.9417
2.0	8.0 x 10 ⁵	95.2941
3.0	2.3 x 10 ⁴	99.8647
4.0	5.0 x 10 ⁴	99.7058
5.0	3.0 x 10 ³	99.9823
6.0	5.0 x 10 ³	99.9705
Control	5.0 x 10 ⁷	0.0

Experimental conditions: Water turbidity = 27 NTU, hardness = 287 CaCO₃ mg/L and pH = 7.1. Total time in full sun = 2 hours.

Table 5 shows the results of testing the effect of temperature at UV 365 nm irradiances of 4, 7 and 11 W/m². The table shows the time in hours when bacterial inactivation reached a reduction of 99.99% when the starting bacterial count was in the order of 10⁵ cells/ml for all tests.

Table 3. Total coliform counts and inactivation percentages at a 17 NTU turbidity water in a static system.

Exposure time (hours)	Total Coliform counts/ 100 ml	Inactivation %
0.0	8.0 x 10 ⁶	0.0
1.0	5.0 x 10 ⁵	93.7500
2.0	1.1 x 10 ⁵	98.6250
3.0	2.2 x 10 ⁴	99.7250
4.0	8.0 x 10 ³	99.9000
5.0	7.0 x 10 ²	99.9912
6.0	3.0 x 10 ²	99.9962
Control	1.3 x 10 ⁷	0.0

Experimental conditions: Water turbidity = 17 NTU, hardness = 293 CaCO₃ mg/L and pH = 7.6. Total time in full sun = 7 hours.

Table 4. Total coliform counts and inactivation percentages at a 10 NTU turbidity water in a static system.

Exposure time (hours)	Total Coliform counts/ 100 ml	Inactivation %
0.0	5.0 x 10 ⁶	0.0
1.0	8.0 x 10 ⁴	98.4000
2.0	2.2 x 10 ⁴	99.5600
3.0	1.1 x 10 ³	99.9780
4.0	2.2 x 10 ²	99.9956
5.0	8.0 x 10 ¹	99.9984
6.0	2.3 x 10 ¹	99.9995
Control	2.8 x 10 ⁶	0.0

Experimental conditions: Water turbidity = 10 NTU, hardness = 276 CaCO₃ mg/L mg/100ml and pH = 7.5. Total time in full sun = 7 hours.

Table 5. Effect of temperature on the inactivation of coliform bacteria by solar UV-365 nm radiation.

Temperature °C	Hours needed for inactivation of 99.99% by UV 365nm irradiance of		
	4.0 W/m ²	7.0 W/m ²	11.0 W/m ²
25.0	7.0	4.0	2.0
30.0	6.0	3.0	2.0
35.0	5.0	2.0	2.0
40.0	4.0	1.0	1.0
45.0	2.0	1.0	1.0

Each number is an average of three readings. Tests were carried out using distilled water with negligible turbidity

3.2. Flow Through Reactor

Figure 1 show the schematic design of the solar flow through disinfection reactor. The original design and the modified system of the reactor performed adequately switching on and off as planned. The flow of water in the original design was uninterrupted and proceeded continuously between the tanks and the reactor and the desired flow rate was adjusted to ensure that maximum exposure time needed to achieve the desired bacterial inactivation is provided. Water flow in the modified system was also flowing in an uninterrupted manner except at situations where high temperature caused flow problems between the solar water heater and the reactor.

Table 6 shows the inactivation results for coliform bacteria using the solar flow through reactor in the form of reduction percentages and exposure times obtained from using contaminated water with coliform bacteria. Table 7 lists the inactivation results of tests on selected bacterial species by means of the solar flow through reactor, and showing the reduction percentages, exposure times needed

to achieve the maximum bacterial inactivation and turbidity of the used water.

Table 6. Inactivation of coliform bacteria by means of solar flow through reactor.

Turbidity NTU	% Inactivation with Time (Hours)			
	3	4	5	6
1.4	98.7	99.9998	-	-
3.0	-	90	99.9998	-
3.5	99.7	99.8	99.9998	-
9.6	92.6	99.8	99.9	99.9998

Table 7. Inactivation of selected bacterial species by means of the solar flow through reactor.

Species	Turbidity NTU	% Inactivation with Time (Hours)			
		1	2	3	4
<i>Staphylococcus</i> spp.	16	99.9998	-	-	-
<i>Salmonella</i> spp.	8.9	99.85	99.9998	-	-
<i>Pseudomonas</i> spp.	2.6	90.0	99.9998	-	-
<i>Streptococcus</i> spp.	5.0	98.8	99.25	99.9998	-
<i>E. coli</i>	2.0	90.3	96.1	99.9	99.9998

The results also indicate that the exposure time needed to reach 99.99% reduction depends on the irradiance of UV radiation and the temperature. The time to obtain this reduction varied between one hour at high UV irradiances, very low turbidity and high temperature to seven hours at low UV radiation and temperature. The average exposure time needed to reach 99.99% reduction at average conditions encountered during testing was six hours. This exposure time decreased rapidly with reduction in the turbidity level and elevation of water temperature.

The results from this study indicate that temperature affects the degree of water disinfection by natural UV radiation. As the temperature of the water increased the time needed to obtain maximum bacterial inactivation is reduced. By increasing the irradiance of solar UV radiation, the time needed to obtain reduction of > 99.99% was reduced compared to a lower UV irradiance at the same temperature. The difference in exposure time required for maximum reductions was not significant for temperatures above 40°C.

4. Discussion

The disinfection of water by UV irradiation has become a credible alternative to chemical disinfection. A number of studies have been conducted to estimate the influence of physical factors, including sunlight and temperature, on the rates of die-off occurring for microbial indicators and pathogens in estuarine and marine waters (Burkhardt III *et al.*, 2000; Davies-Colley *et al.*, 1994; Fujioka *et al.*, 1981; Kapuscinski and Mitchell, 1983; Rippey *et al.*, 1987). The results from this study obtained in the laboratory scale indicate that turbidity is a factor that influences the reduction percentage of bacteria and thus the efficiency of UV disinfection of contaminated water. At turbidity higher than 5 NTU, the efficiency starts decreasing rapidly until it reaches approximately 93%. To reach higher efficiency percentages, it is necessary to increase the exposure time.

It is worth mentioning that the Jordanian national standards for drinking water state that the acceptable range for turbidity is less than or equal to 5 NTU. Thus, in cases where drinking water exceeds the Jordanian standards, it is necessary to physically treat the water by filtration methods which are efficient in decreasing turbidity levels prior to disinfecting it by ultraviolet. Furthermore, the results indicate that the efficiency increases with increased exposure time, in other words, at high turbidity conditions and severe weather conditions, it is necessary to increase the exposure time to compensate for the effects of these factors. Furthermore, the results were used to adjust the flow rate of water in the reactor to ensure that water in the reactor is exposed to at least five hours of UV radiation before leaving the reactor to the storage tanks.

The effects of solar radiation, temperature, salinity, and other factors on the survival of *Salmonella typhimurium* (Smith *et al.*, 2000), *Escherichia coli*, *Clostridium perfringens*, and male – specific bacteriophage in estuarine waters have been studied (Burkhardt III *et al.*, 2000). Temperature influenced the lethal effects from solar radiation only for fecal coliforms and the inactivation of fecal coliforms was significantly and inversely related to temperature and positively related to accumulated light energy (Burkhardt III *et al.*, 2000). Joyce *et al.*, (1996) studied water samples heavily contaminated with *E. coli* and heated to temperatures of 50, 55, and 59.5°C and exposed to full Kenyan sunshine (maximum water temperature, 55°C). No cultivable *E. coli* organisms were detected at either the end of the experiment or a further 12 hours later.

There have been several reports about the influence of lamp intensity and water transmittance on the UV disinfection of water ((Blatchley III and Hunt, 1994; Sommer and Cabaj, 1993; Sommer *et al.*, 1997; Sommer *et al.*, 1999). The influence of fluence distribution on the result of biosimetry was investigated by Cabaj *et al.* (1996). In the single lamp pilot system, UV lamp intensity reduced the reduction equivalent dose more than water transmittance (Sommer *et al.*, 1999).

The UV irradiance measured during the summer season and between 10:00 A.M. and 3:00 P.M. ranged between 23 - 27 W/m² indicating an ample level of UV irradiance to carry out water disinfection. The UV irradiance decreases during the winter season and cloudy or rainy conditions to about 4 W/m² which is still sufficient to carry out water disinfection at low levels of turbidity. Burkhardt III *et al.*, (2000) showed that light energy was greatest during the summer in Alabama trials and the greatest increase of accumulated light energy occurred between 4 and 8 hours after sunrise [11 A.M-3 P.M.]; this accounted for 55-58% of the total energy observed. The results indicate that *E. coli* needed four hours to reach 99.9998 % reduction, which was the longest time for all bacterial species tested, indicating that it is less sensitive to UV radiation than other tested bacterial types. Thus, it can be used as an indicator for the efficiency of UV disinfection of water. *Staphylococcus* spp. was the most susceptible species to UV disinfection and needed only one hour of exposure time even at high turbidity level (16 NTU). It is worth mentioning that future research should include in the evaluation of the efficacy of disinfection plants using additional test organisms such as bacterial spores or

bacteriophages. This is due to the fact that although the more UV sensitive indicator bacteria are no longer detectable, other more UV resistant pathogens could be still present in the water.

The first design of the reactor was successful in inactivating the bacteria tested although it suffered from one drawback, which is the need for electrical power (minimal power to drive a 6 watt submersible pump). Therefore, all rural areas where electricity is not easy available or absent will not be able to use the reactor, which explains the need for the modified version of the reactor. The modification of the reactor to include a flat solar water heater, heat exchanger and a thermal one-way valve eliminated the need for the pump to circulate the water and thus for electrical power. On the other hand, the design faced flow problems specially at elevated temperatures mainly in the form of reduction in flow rates and sometimes back flow problems. Thus, the exposure times needed to achieve the required water disinfection had to be changed slightly by decreasing the flow rate of water through the reactor. This adjustment of flow rate provided additional exposure time. Further modifications of the design are needed to solve the flow problems. Furthermore, the long term stability of the PET used for the first reactor with respect to a sufficient UV transmittance should be the subject of future studies. Another point to be included in future studies is the influence of the spectral UV absorption of the water.

This investigation showed that, based on the bacterial species examined, water turbidity is a major factor influencing water disinfection by natural UV radiation and that on increased exposure time or filtration methods are needed to reach maximum bacterial inactivation. Furthermore, temperature increased the level of bacterial inactivation. It appears that the temperature functions as an accelerating factor in bacterial inactivation when applying UV radiation. Further investigations are needed to determine the exact mechanism of temperature accelerated bacterial inactivation, to improve the design of the solar water disinfection reactor and to test its performance with regard to more resistant microorganisms like bacterial spores and viruses.

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Spatial and Seasonal Variations in Biomass and Size Structure of Zooplankton in Coastal Waters of the Gulf of Aqaba

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Abstract

Zooplankton biomass were measured monthly from eleven coastal sampling station and one offshore station from the northern part of the Gulf of Aqaba between January and December 2004. Significant difference in zooplankton biomass was obtained among different stations and different sampling months. The highest biomass was recorded in January (50.75 mg. dry wt.m⁻³), the lowest in October (5.54 mg. dry wt.m⁻³). A notable high biomass of 46.1 mg. dry wt.m⁻³ was registered at Tala Bay pool, while lowest biomass was recorded at both Al-Sodasiat and offshore stations. At the study area, the results showed significant differences among different size fractions of zooplankton. The relative annual average of size fraction > 500 µm constituted about 68 % of the total biomass, while the size fractions 200-500 µm and 100-200 µm constituted 26% and 6%, respectively.

المخلص

تم التقدير الحجمي للهوائيم الحيوانية في خليج العقبة ما بين كانون الثاني- كانون أول ٢٠٠٤ في إحدى عشر موقع ساحلي و موقع في عرض البحر. دلت النتائج على وجود فروق معنوية بين المناطق المختلفة و كذلك بين الأوقات المختلفة. سجلت الدراسة تركيز عالية بلغت ٥٠,٧٥ ملغ / م^٣ في شهر كانون الثاني بينما سجلت تركيز منخفضة خلال شهر تشرين أول، وقد دلت كذلك النتائج على وجود تركيز عالية في بحيرة النخيل و تركيز منخفضة في منطقة السداسيات و الشاطئ العام في الجهة الجنوبية من الخليج. بينت النتائج وجود فروق معنوية بين الأحجام المختلفة للهوائيم الحيوانية حيث بلغت نسبة الحجم الأكبر من ٥٠٠ ميكرون حوالي ٦٨ % يتبعها ٢٦ % للحجم من ٢٠٠-٥٠٠ ميكرون، أما نسبة الحجم من ١٠٠-٢٠٠ ميكرون فقد بلغت ٦ % فقط.

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Keywords: Zooplankton; biomass; size fraction; Gulf of Aqaba;

1. Introduction

The Gulf of Aqaba extends for about 180 km, the Jordanian portion of Gulf of Aqaba extends for 27 km only. Along the Jordanian coast and within the present study area the Gulf reaches a maximum width of around 25 km while in the northern half the maximum width is only around 17 km and it narrows to about 5 km at the most northern tip. The maximum depth of the Gulf is about 1830 m while the average depth is around 650 m (Marcos 1970; Levanon-Spanier *et al.*, 1979).

The water in the study area is very clear with very high transparency under normal conditions. Many human activities are taking place along the Gulf and many development projects exist or are planned, particularly at the northern tip of the Gulf. There are signs that these activities are affecting zooplankton biomass indirectly by increasing the nutrient availability in water, which leads to an increase in the plankton biomass in the area (Al-Najjar

and Rasheed, 2005). Zooplankton organisms as community are important grazers in the pelagic zone (Burkill *et al.*, 1993; Landry *et al.*, 1995), consuming a wide range of food particles that vary in size and type. The zooplankton also aids in the vertical export of carbon out of the mixed layer as particulate, skeletal, and fecal material (Roman *et al.*, 1995; Stoecker *et al.*, 1996). Within the zooplankton community, organisms of different size may play different roles in biogeochemical cycling and so influence processes associated with the biological mediated drawdown of atmospheric CO₂, and its transformation into particles and sedimentation in the ocean.

In the Gulf of Aqaba, quantitative data sets on the seasonality of zooplankton abundance and biomass distribution have been presented by several investigations since 1970 (Gordeyeva 1970; Vaissiere and Sequin 1982; Echelman and Fishelson 1990; Al-Najjar 2000; Al-Najjar *et al.*, 2002 and Al-Najjar 2004a). The objective of the present work was to test temporal and spatial variations

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and abundance of zooplankton biomass of different fraction sizes in the coastal waters of the Gulf of Aqaba.

2. Materials and Methods

Surface zooplankton samples were collected from eleven coastal stations along the northern tip of the Gulf of Aqaba [Wahat Ayla, Hotels, Public cafes, Phosphate port, Clinker, Marine Science Stations (MSS), Public beach, Al-Sodasiat, Tala bay (inside), Tala bay (outside) and Jordan Fertilizer Industry site (JFI)] and one offshore station located about 2 Km from the coast line (Fig. 1).

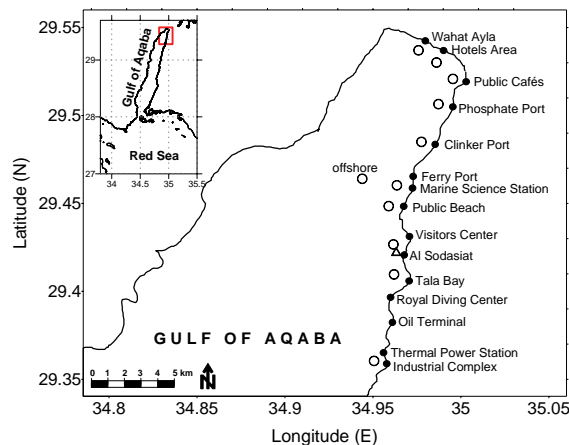


Fig. 1: Zooplankton sampling stations along the northern part of the Gulf of Aqaba

The depth of coastal stations ranging between 4 and 50 m, while the offshore station has depth of about 200-300 m. Zooplankton $>100 \mu\text{m}$ was sampled monthly between January - December 2004 during daylight. A simple plankton net (100 μm mesh; ARI, USA) was towed horizontally from a boat at a speed of 3-3.5 knots for duration of ten minutes along the surface. Zooplankton samples were kept on ice for about 2 hours until arrival to the laboratory. In the laboratory, samples were size-fractionated using a column of 100, 200 and 500 μm mesh filters. The separated fractions were filtered on pre-dried and pre-weight GF/C filters, dried for 24-48 hrs at 60 °C, and re-weighed. Biomass (mg. dry wt. m^{-3}) was calculated as follows:

$$\text{Biomass (mg.l}^{-1}\text{)} = \left[\frac{\text{zooplankton dry weight (gm)}}{\text{volume of water filtrate (m}^{-3}\text{)}} \right] \times 1000,$$

Where the volume of water filtrate = velocity (m. sec^{-1}) \times area of net (m^2) \times time of collection (sec). The effect of 'station' (n=12) and 'month' (n=12) on zooplankton biomass was tested statistically using an ANOVA test. The statistical significance was tested at the 95% confidence level.

3. Results

Throughout the area of study, zooplankton biomass was characterized by relatively low values ranging between 0.462 and 4.22-mg. dry wt. m^{-3} with an annual average of

18.40 mg. dry wt. m^{-3} . The spatial distribution of zooplankton biomass showed clear variations between different sampling stations (Fig. 2). The maximum biomass value of 46.1 mg. dry wt. m^{-3} appeared at Tala Bay pool (inside), while lowest was obtained at offshore station (Fig. 2). Another notable high biomass values were also registered at Hotel and Wahat Ayla stations, which located at the most northern part of the Gulf (21.8 and 22.62 mg. dry wt. m^{-3} , respectively). Statistically, a significant difference in zooplankton biomass was obtained between different stations ($P=0.0028$).

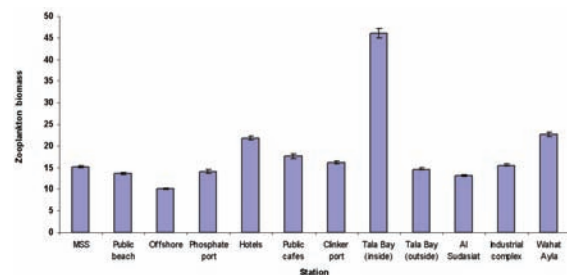


Fig 2: Average spatial variations of zooplankton biomass (mg. dry wt. $\text{m}^3 \pm \text{SE}$) at different sampling stations

The monthly average of zooplankton biomass varied throughout the year fluctuating between 0.842 and 3.842 mg. dry wt. m^{-3} at stations offshore and Tala Bay (inside), respectively. On a monthly scale, January exhibited the highest biomass value (average 50.75 mg. dry wt. m^{-3}). Other small increases of biomass appeared in February, March and April with values of 20.4, 28.92 and 30.30 mg. dry wt. m^{-3} , respectively (Fig. 3). On the other hand, the lowest zooplankton biomass was detected during summer and early autumn with a minimum biomass of 5.54 mg. dry wt. m^{-3} in October. The results of ANOVA test showed a high significant variation in the zooplankton biomass between different sampling months ($P < 0.0001$).

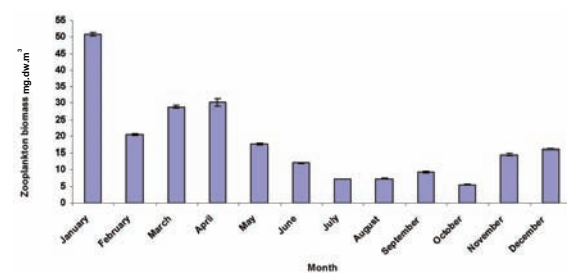


Fig 3: Average monthly variations in zooplankton biomass (mg. dry wt. $\text{m}^3 \pm \text{SE}$) during the period of study

During the period of study, at all sampled stations the size fraction $>500 \mu\text{m}$ dominate biomass followed by 200-500 μm and 100-200 μm size fraction (Fig. 4). The relative contribution of each size fraction to the total biomass showed that about 67% of the biomass was due to organisms more than 500 μm , while 26 % and 7 % due to the organisms less than 200-500 μm and 100-1200 μm fractions, respectively (Table 1). High statistically significant difference in zooplankton biomass was obtained values between different size fractions ($P < 0.0001$) (Fig. 5).

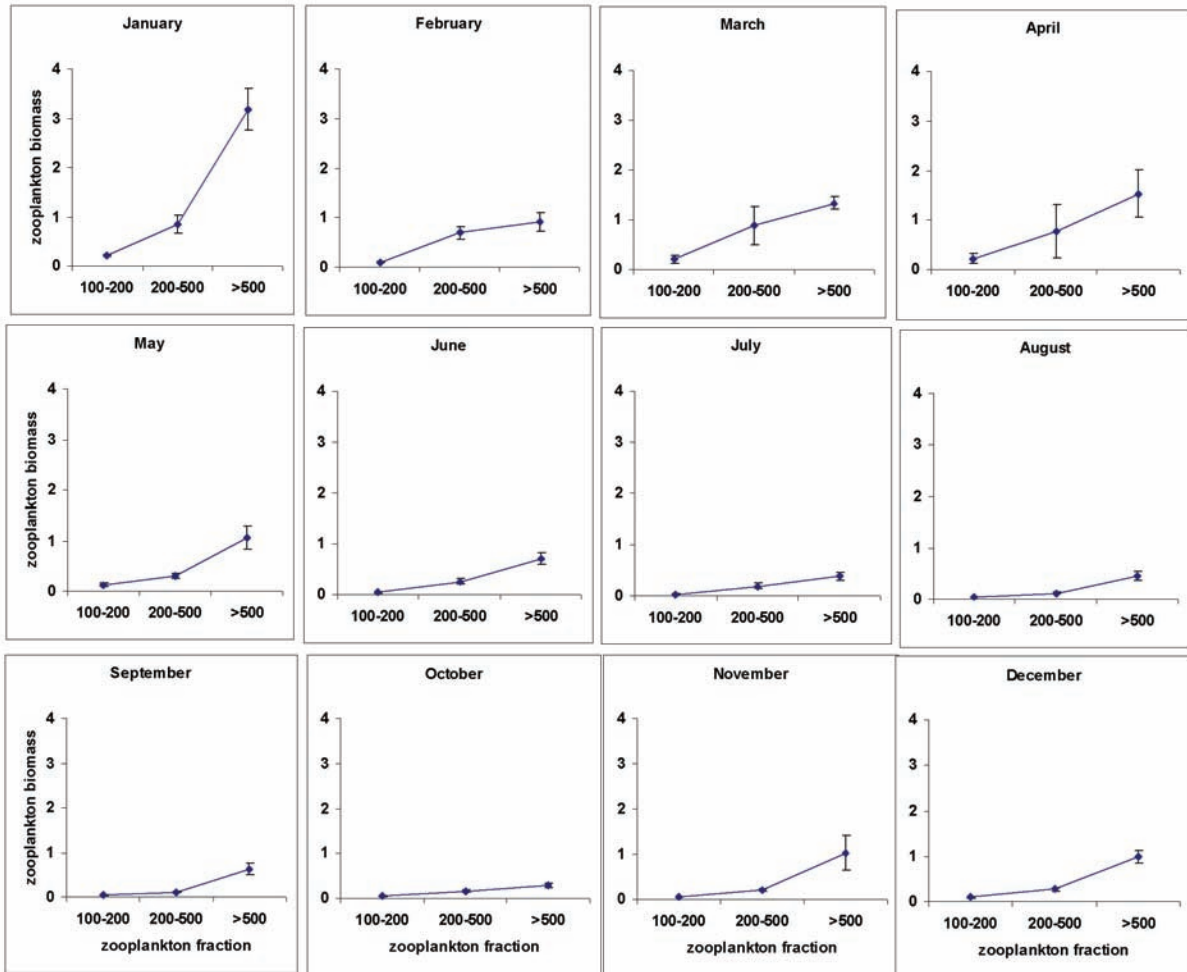


Fig. 4: Monthly variations of the average biomass of different zooplankton size fractions (mg. dry wt. m³ ± SE) during the period of study

Table 1: Mean biomass (mg. dry wt. m³) and percentage of zooplankton different size fractions in the Gulf of Aqaba between January and December 2004. (SE) standard error, (N) number of fraction repetitions.

Fraction (um)	Biomass ± SE	Percentage	N
100	13.20 ± 0.20	6 %	12
200-500	55.30 ± 0.86	26 %	12
>500	144.55 ± 1.25	68 %	12
Total	213.05	100 %	36

4. Discussion

The oligotrophic marine areas are characterized by both low primary and secondary production. The annual average of zooplankton biomass (18.40 mg. dw. m⁻³) during the present study was obviously lower than other previous studies of Al-Najjar *et al.* (2002) and Al-Najjar (2004b) at the same area (25.53 and 71.26 mg. dw. m⁻³, respectively). The lower difference in zooplankton biomass obtained is likely due to different sampling strategy (Avois *et al.* 2000). However, the obtained values during this study fall within the range of other oligotrophic water (steifox *et al.* 1999). The highest zooplankton biomass of 46.1-mg. dry wt.m⁻³ recorded at Tala Bay pool (inside) station could be attributed to its semi-enclosed characteristics and shallowness (4 m). Additionally, this

site is characterized by higher annual average chlorophyll contents of 0.66 mg/m³ compared with other stations (0.30 mg/m³) (MSS, 2006). Generally, spatial and temporal variations in zooplankton biomass throughout the northern Gulf of Aqaba can be related to variations in the phytoplankton production cycles as observed in other marine habitats including Indian Ocean (Piontkovski *et al.*, 1995; Haury 1988).

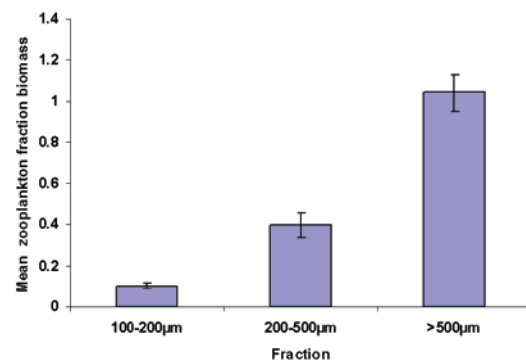


Fig. 5: Mean biomass of zooplankton different size fractions in the study area collected during 2004

In this study, consumer community of zooplankton seems follow autotrophic biomass evolution. The peak of

zooplankton biomass was highest in winter following the annual peaks of phytoplankton in the Gulf of Aqaba where there is an increase in eukaryotic algae during the period of January to February and diatom abundance at the beginning of March (Al-Najjar 2000; Cornils *et al.*, 2005). Similar finding have been observed by Mozetic *et al.*, (1998) in the Gulf of Trieste during his study from 1989 to 1995. Also this higher biomass in winter could be explained by the dominance of the larger sized zooplankton organisms such as copepods (especially *Calanus*, *Candacia* and *Centropages* and *Clausocalanus*), urochordates, cheatognaths and mollusk larvae (Cornils *et al.*, 2005; Personal observation). On the other hand, the lower peak of zooplankton biomass during summer is possibly due to thermal stratification in the Gulf of Aqaba, where nutrient concentration are also low (Lindell and post 1995; Al-Najjar 2000; Cornils *et al.*, 2005; Al-Najjar *et al.*, 2007). In this condition, there was a decrease in eukaryotic algae and diatoms abundance, which lead to a decrease in density of zooplankton biomass. Another explanation pending on the thermal stratification during summer concurrent with low nutrient availability, whereas during winter mixing season water becomes homogenized in such condition there was an increase in eukaryotic algae abundance, which leads to an increase in biomass of zooplankton organisms. It has been shown that environmental variability plays a major role in determining spatial and temporal patterns of zooplankton distribution (Tomosada and Odate 1995; Aoki, *et al.*, 1990). In General, zooplankton abundance has been associated with changes in phytoplankton standing stocks (Al-Najjar 2000; Al-Najjar *et al.*, 2002) and with combined effects of regional climatology and local hydrographic variables Aoki *et al.*, (1990).

Body size has also been considered a specific component of plankton heterogeneity from small protozoan to large copepods in the oligotrophic waters such as Gulf of Aqaba (Sommer *et al.*, 2002). Differences were also observed in the biomass levels in the different size class studied, where about 68 % of the biomass was observed to be in the large size fraction >500 μm , and size fraction of 100-500 μm accounted for only 32%. In comparison with the result of polar water, the small size fraction accounted for about 50 % of total biomass while the largest one represented 35% (Hernandez-Leon *et al.*, 2000). The reason is due to copepods in cold water accounted 50-90 % of the total zooplankton number Conover and Huntley (1991) and they are in small size and are, therefore, better contributors to the energy flow in these cold waters Boyson-Ennen *et al.*, (1991). However, another study by Hernandez-Leon *et al.*, (2000) for the same area and same size fraction of zooplankton during January 1993, found that the zooplankton biomass was very low (64.31 mg. dw. m^{-2}), and about 56 % of total biomass was due to the large size fraction 1000-4000 μm while the smallest one 200-500 μm accounted for about 26 %. Those results could reflect the interannual variability but also the progression of the different populations from spring to summer. This would imply that organisms have growth rates, which might reach higher values during the productive season. Predation by planktivorous fish explains the smaller spatial heterogeneity in this study, since the predation pressure seems to be an essential variable in the

maintenance of horizontal pattern in zooplankton distribution (Gliwicz and Rybowska, 1992). Zooplanktivorous fish account for about 70% of the total fish species registered in the Gulf of Aqaba (Khalaf and Kochzius, 2002). Among the zooplanktivorous fish, two species (*Pseudonthias squamipinnis* and *Neopomacentrus miryae*) occur in great numbers in the study area (Khalaf and Kochzius, 2002).

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Heavy Metals in Three Commonly Available Coral Reef Fish Species From the Jordan Gulf of Aqaba, Red Sea

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Abstract

The concentrations of the heavy metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were determined in the muscles, livers, gills, gonads, and the stomachs of two detritus feeder (*Ctenochaetus striatus* and *ZebraSoma xanthurum*) and one herbivorous (*Scarus ferrugineus*) fish species collected from the Gulf of Aqaba. The mean concentrations of heavy metals among the organs of fish examined were generally significantly different ($P < 0.05$). In general, muscles contained lower metal concentrations than other organs. Comparing species, there was no significant difference in the mean concentration of metals except Zn in the muscles of the examined fish species. By comparison, the mean concentrations of Zn, Cd and Cu were significantly different among the livers of these species. In gills, only Mn varied significantly. Only Cu and Pb varied among the stomachs of species examined. In gonads, there were significant differences between the mean concentrations of Cd, Cr, Cu, Mn, and Zn in one of the detritus feeder fishes and the herbivorous fish. The concentration of metals in the muscle tissues were generally low and within the ranges expected for metals in muscle of fish from relatively uncontaminated locations. The ranges are generally lower or within the ranges of the concentrations for these elements in fishes of the Red Sea (Hanna, 1989). Moreover, the values fall below the acceptable levels for human consumption recommended by FAO (1983) and WHO (1978, 1989, 1993) which means that they do not pose a significant threat to the health of human consumers. The results indicate that relatively high concentrations of heavy metals were found in liver and gill of the examined species, which suggest the possibility of using these two organs, particularly the liver, as bioindicators of metals present in the surrounding environment.

المخلص

تم تعيين تراكيز العناصر الثقيلة الكاديوم والكروم والنحاس والحديد والمنجنيز والنيكل والرصاص والزنك في عضلات وكبد وخياشيم ومناسل ومعدة لنوعين من الأسماك الرمية (كتينوكتيتاس ستراتس و زيبراسوما كزانثورام) ونوع من الأسماك اكلة الأعشاب (سكارس فيروجينيوس) جمعت من خليج العقبة. كانت هنالك فروقات إحصائية في تراكيز العناصر الثقيلة بين اعضاء الأسماك التي تم فحصها. وبشكل عام احتوت العضلات على أقل تراكيز للعناصر الثقيلة. بمقارنة أنواع الأسماك لم تكن هنالك فروقات إحصائية في تراكيز العناصر باستثناء الزنك في العضلات. بمقارنة تراكيز الزنك والكاديوم والنحاس في كبد السمك كان هنالك فروقات إحصائية. أما تراكيز المنجنيز فكانت هنالك فروقات إحصائية في الخياشيم. وأختلف كذلك إحصائيا تراكيز النحاس والرصاص في معد الأسماك. أما في المناسل فكانت تراكيز الكاديوم والكروم والنحاس والمنجنيز والزنك تختلف إحصائيا. كانت تراكيز العناصر في العضلات منخفضة وضمن التراكيز المتوقعة للمناطق غير الملوثة. تقع هذه التراكيز ضمن مدى التراكيز لمنطقة البحر الأحمر وضمن التراكيز المقبولة للأستهلاك البشري حسب منظمة الغذاء الدولية ومنظمة الصحة العالمية. وتشير التراكيز العالية للعناصر في الخياشيم و الكبد بإمكانية إستخدام هذه العناصر كمؤشر حيوي عن تراكيز العناصر في البيئة المحيطة.

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Keywords: Heavy metals; Coral reef fishes; Herbivorous fish; Detritus feeder fish; Gulf of Aqaba; Red Sea;

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1. Introduction

In recent years, the concentration of heavy metals in marine fishes has received great attention, particularly in developed and industrialized coastal areas (Denton and Burdon-Jones, 1986; Wahbeh and Mahasneh, 1987; Hanna, 1989; Rayment and Barry, 2000; Miao, 2001). Since 1975 the shipping and industrial activities at the Jordanian Aqaba town at the northern side of the Gulf of Aqaba have been increased tremendously (Abu Hilal, 1999). Parallel to this there has been a great demand for coastal development. The following categories of human activities are of particular environmental concern with regard to maintaining healthy coral reefs and pollution problems in the Gulf of Aqaba: tourism, sport and commercial fishing, boat anchoring, shipping of oil and hazardous material, dumping of debris and litter, wastewater disposal, and mariculture. Industrial and commerce activities in the Gulf have been increased with development of oil, marina, phosphate mineral and potash export and other industrial facilities. Shipping traffic in the northern Gulf of Aqaba is expected to increase during the next decade. Tourism and population growth is expected to continue during the next two to three decades, due in part to governmental policies encouraging growth in the area and facilitating increased tourism infrastructure. Consequently, unfavorable impact of these activities on the marine life is to be expected. A number of positive steps have been taken to address some of these concerns, however, many problems remain and expected to increase as both the permanent and tourist population growth. During the past two decades, several authors have studied the composition of heavy metals in the marine environment of the Gulf of Aqaba (Abu-Hilal, 1987; Abu-Hilal and Badran, 1990; Abu-Hilal *et al.*, 1993). Abu-Hilal (1987) has reported abnormally high concentrations (3-9 times) of heavy metals in near shore surface sediments at some stations in the northern portion of the Gulf of Aqaba. Moreover, the metal content of sea grasses (Wahbeh, 1984; Abu-Kharma, 2006), algae (Wahbeh *et al.*, 1985; Abu-Kharma, 2006), crustacea (Abu-Hilal *et al.*, 1988), zooplankton (Bani-Fawwaz, 2005), mussels (Ababneh, 2004; Al-Bataineh, 2004), corals (Al-Shloul, 2006; Al-Tarabeen, 2006) and in several fish species (Wahbeh, 1985; Wahbeh and Mahasneh, 1987) have been reported. However, the amount of work on heavy metals in marine biota of the Gulf of Aqaba is still limited and there is a need for more studies. The lack of integrated data and scientific assessment of pollutants, their sources and effects on coral reef resources in the Gulf has impacted and hindered the management actions and efforts made by related authorities in this region (Abu Hilal, 1999; RSMPP, 2003). The present paper provides important information on the metal content in the muscles, livers, gills, gonads, and the stomachs of two detritus feeder fish species, *Ctenochaetus striatus* and *Zebrasoma xanthurum* and one herbivorous fish species *Scarus ferrugineus* that add to the limited scientific data and may be useful for environmental managers.

2. Materials and Methods

A total of 22 specimens of fish, 11 of the lined bristle tooth surgeonfish *C. striatus*, 4 of the yellow surgeonfish *Z. xanthurum*, and 7 of the parrot fish *S. ferrugineus*, were collected from three coastal areas along the northern portion of the Gulf of Aqaba (Fig. 1).

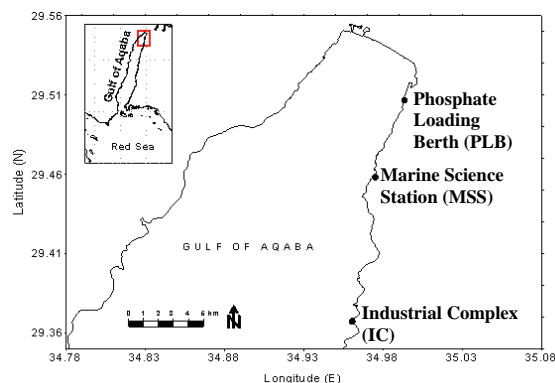


Figure 1: Locations of the sampling sites

The samples were collected using set-nets positioned at site, fish taps and spear gun. Spear shafts and heads were made of seawater resistant, stainless steel there by minimizing sample contamination. After capture the samples were weighed, measured, cleaned deionized-distilled water, stored in pre-cleaned plastic a, and kept frozen at -18°C until further analysis. Pretreatment, preparation of sub samples and analysis were made according to FAO Technical Paper No. 212 *rrPC3* (1983). Frozen fish were partially thawed and dissected on a cleaned plastic sheet using scalpels with steel blades and plastic forceps. Flesh, liver, gill, gonads and stomach were taken out and dried in a pre-cleaned glass container at 80°C to a constant weight.

A suitable volume of a mixture of hydrogen peroxide/nitric acid solution 1:1 v/v was used for the wet acid digestion of a pre-weighed tissue or organ. The analyses of all metals were performed by flame atomic spectrometry using a Perkin Elmer 3030 atomic absorption spectrophotometer with digital read-out, deuterium lamp background correction, and automatic zero to compensate the blank. Settings were those recommended by the manufacturer.

A standard curve was run with each analysis. A blank treated exactly as for the sample was also run with each batch of samples. Blanks were always of negligible values. The effect of interferences attributable to the matrix and the validity of the results were checked with the standard addition method. Recoveries were between 98 and 103%. The precision was confirmed by carrying out ten replicate analyses for three different samples. The coefficient of variation was less than 5% for all elements. Detection limits in $\mu\text{g g}^{-1}$ (ppm) were 0.003 for Cd, 0.004 for Cr, 0.007 for Co, 0.003 for Cu, 0.005 for Fe, 0.003 for Mn, 0.008 for Ni, 0.005 for Pb and 0.002 for Zn. The AVOVA and Student's-t comparison tests have been used to compare the mean concentrations of heavy metals in different fishes and organs of these selected fish species.

3. Results

The mean concentrations of metals ($\mu\text{g g}^{-1}$ dry weight) in the muscles, livers, gonads, gills, and the stomachs of fish examined are summarized in Tables 1, 2, 3, 4, and 5, respectively. The analysis of variance showed that the mean concentrations of heavy metals among the organs of each fish species were significantly different ($P < 0.05$), except for Cr in the three species, Fe in *C. striatus*, and Mg in *Z. xanthurum* (Table 6). In general, the muscles of fish examined contained lower mean concentrations of metals. Among species, however, there were no significant differences ($P > 0.05$) in the mean concentrations of metals in muscles, except for Zn where the mean ranged from $10.61 \mu\text{g g}^{-1}$ in *S. ferrugineus* to $21.38 \mu\text{g g}^{-1}$ in *C. striatus*. Similarly, there was a wide and significant difference in the mean concentrations of Zn among the livers of the three species, where it ranged from $82.78 \mu\text{g g}^{-1}$ in *S. ferrugineus* to $1512.57 \mu\text{g g}^{-1}$ in *C. striatus*. Moreover, there were significant differences in the means of Cd ($1.86 \mu\text{g g}^{-1}$ in *S. ferrugineus* to $10.86 \mu\text{g g}^{-1}$ in *C. striatus*) and Cu ($9.91 \mu\text{g g}^{-1}$ in *S. ferrugineus* to $67.28 \mu\text{g g}^{-1}$ in *Z. xanthurum*) among the livers of these fishes. Among the gills of the examined fish species, significant differences were found in the mean concentrations of Mg and Mn.

Table 1: Concentrations (Mean \pm S.D $\mu\text{g g}^{-1}$ dry weight) of metals in the muscles of three species of fish from the Gulf of Aqaba, Red Sea.

Metal	<i>Ctenochaetus striatus</i>	<i>Zebrasoma xanthurum</i>	<i>Scarus ferrugineus</i>	Difference among species
Cd	0.83 \pm 0.28	0.54 \pm 0.29	0.61 \pm 0.22	NS*
Co	2.23 \pm 0.94	2.88 \pm 1.24	2.29 \pm 0.64	NS
Cr	1.46 \pm 1.01	3.03 \pm 2.73	1.86 \pm 1.82	NS
Cu	0.87 \pm 0.47	1.03 \pm 0.62	1.26 \pm 1.35	NS
Fe	8.47 \pm 8.76	12.46 \pm 12.06	8.50 \pm 8.07	NS
Mg	892 \pm 389	960 \pm 306	951 \pm 354	NS
Mn	0.93 \pm 0.39	1.03 \pm 0.54	0.93 \pm 0.35	NS
Ni	1.90 \pm 0.71	2.53 \pm 1.03	2.17 \pm 1.90	NS
Pb	4.05 \pm 1.24	5.27 \pm 1.18	4.23 \pm 3.13	NS
Zn	21.38 \pm 9.68	18.58 \pm 17.05	10.61 \pm 2.45	S**

* NS: Not significant ($P > 0.05$); ** S: Significant ($P < 0.05$).

Table 2: Concentrations (Mean \pm S.D $\mu\text{g g}^{-1}$ dry weight) of metals in the livers of the examined fish species from the Gulf of Aqaba, Red Sea. Abbreviations are as in Table 1

Metal	<i>Ctenochaetus striatus</i>	<i>Zebrasoma xanthurum</i>	<i>Scarus ferrugineus</i>	Difference among species
Cd	10.86 \pm 9.12	5.68 \pm 5.06	1.86 \pm 1.52	S
Co	7.12 \pm 8.60	4.70 \pm 3.75	3.69 \pm 5.02	NS
Cr	2.16 \pm 1.76	2.18 \pm 0.88	2.74 \pm 2.04	NS
Cu	45.36 \pm 29.02	67.28 \pm 67.74	9.91 \pm 11.12	NS
Fe	2087 \pm 1832	1575 \pm 1344	723 \pm 522	NS
Mg	1163 \pm 1344	883 \pm 367	1059 \pm 1019	NS
Mn	5.55 \pm 4.84	3.85 \pm 0.49	3.41 \pm 1.74	NS
Ni	4.83 \pm 4.56	2.95 \pm 1.64	4.41 \pm 4.95	NS
Pb	14.54 \pm 13.46	6.95 \pm 2.70	8.02 \pm 12.33	NS
Zn	1512 \pm 897	222 \pm 227	82.78 \pm 66.72	S

Table 3: Concentrations (Mean \pm S.D $\mu\text{g g}^{-1}$ dry weight) of metals in the gonads of the examined fish species from the Gulf of Aqaba, Red Sea. Abbreviations are as in Table 1.

Metal	<i>Ctenochaetus striatus</i>	<i>Zebrasoma xanthurum</i> *	<i>Scarus ferrugineus</i>	Difference among species
Cd	0.88 \pm 0.79	0.25	2.19 \pm 1.51	S
Co	3.05 \pm 2.14	2.50	12.70 \pm 17.84	NS
Cr	1.18 \pm 0.74	0.50	9.69 \pm 10.80	S
Cu	3.10 \pm 0.92	2.00	7.08 \pm 4.97	S
Fe	101.76 \pm 72.31	22.50	83.68 \pm 61.06	NS
Mg	910 \pm 616	185	1267 \pm 1177	NS
Mn	2.35 \pm 1.58	2.00	5.96 \pm 2.78	S
Ni	3.52 \pm 4.41	1.50	4.76 \pm 2.63	NS
Pb	3.63 \pm 1.68	2.50	11.53 \pm 14.64	NS
Zn	243.60 \pm 183.28	155.00	506.22 \pm 227.2	S

* only one specimen; ** difference between *C. Striatus* and *S. ferrugineus*

Table 4: Concentrations (Mean \pm S.D $\mu\text{g g}^{-1}$ dry weight) of metals in the gills of the examined fish species from the Gulf of Aqaba, Red Sea. Abbreviations are as in Table 1

Metal	<i>Ctenochaetus striatus</i>	<i>Zebrasoma xanthurum</i>	<i>Scarus ferrugineus</i>	Difference among species
Cd	2.19 \pm 0.54	2.21 \pm 0.82	1.63 \pm 0.57	NS
Co	11.73 \pm 3.30	10.76 \pm 5.34	10.33 \pm 4.42	NS
Cr	4.41 \pm 2.27	8.03 \pm 7.92	5.39 \pm 4.01	NS
Cu	3.82 \pm 1.47	3.65 \pm 1.11	7.92 \pm 0.52	NS
Fe	105.79 \pm 34.52	128.07 \pm 74.26	173.93 \pm 97.53	NS
Mg	1050 \pm 621	1037 \pm 345	2115 \pm 1335	S
Mn	6.49 \pm 1.91	7.46 \pm 2.69	22.33 \pm 6.73	S
Ni	7.97 \pm 2.78	6.99 \pm 3.04	6.19 \pm 2.59	NS
Pb	19.47 \pm 7.52	22.24 \pm 13.19	14.41 \pm 6.12	NS
Zn	51.32 \pm 21.11	46.63 \pm 21.74	46.29 \pm 15.33	NS

Table 5: Concentrations (Mean \pm S.D $\mu\text{g g}^{-1}$ dry weight) of metals in the stomachs of the examined fish species from the Gulf of Aqaba, Red Sea. Abbreviations are as in Table 1

Metal	<i>Ctenochaetus striatus</i>	<i>Zebrasoma xanthurum</i>	<i>Scarus ferrugineus</i>	Difference among species
Cd	4.13 \pm 2.34	2.95 \pm 1.61	4.92 \pm 3.86	NS
Co	23.68 \pm 13.32	16.00 \pm 10.23	24.01 \pm 19.68	NS
Cr	9.06 \pm 13.49	17.98 \pm 16.39	17.29 \pm 27.37	NS
Cu	6.41 \pm 2.51	11.43 \pm 5.40	11.24 \pm 6.13	S
Fe	5150 \pm 1123	2080 \pm 1291	1431 \pm 1785	NS
Mg	5620 \pm 2397	8409 \pm 9584	5310 \pm 1752	S
Mn	45.73 \pm 34.41	48.75 \pm 39.96	27.87 \pm 23.95	NS
Ni	15.31 \pm 9.34	13.75 \pm 7.72	16.64 \pm 14.58	NS
Pb	41.32 \pm 24.08	22.35 \pm 20.70	14.41 \pm 6.12	S
Zn	91.76 \pm 34.47	105.00 \pm 102.67	99.34 \pm 22.73	NS

The highest Mg mean concentration found was $2115 \mu\text{g g}^{-1}$ in the gills of *S. ferrugineus* and the lowest ($1037 \mu\text{g g}^{-1}$) in *Z. xanthurum*. Similarly, Mn was highest in *S. ferrugineus* ($22.33 \mu\text{g g}^{-1}$) and the lowest in *C. striatus* ($6.49 \mu\text{g g}^{-1}$). Among the stomachs of various species, there were significant differences in the mean concentrations of Mg, Cu, and Pb. The mean concentration of Mg ranged from $5310 \mu\text{g g}^{-1}$ in *S. ferrugineus* to 8409

$\mu\text{g g}^{-1}$ in *Z. xanthurum*. The mean concentration of Cu ranged from $6.41 \mu\text{g g}^{-1}$ in *C. striatus* to $11.43 \mu\text{g g}^{-1}$ in *Z. xanthurum*, while Pb ranged from $14.41 \mu\text{g g}^{-1}$ in *S. ferrugineus* to $41.32 \mu\text{g g}^{-1}$ in *C. striatus*. In gonads, there were significant differences ($P < 0.05$) student t-test) between the means of Cd (0.88 and $2.19 \mu\text{g g}^{-1}$), Cr (1.18 and $9.69 \mu\text{g g}^{-1}$), Cu (3.10 - $7.08 \mu\text{g g}^{-1}$), Mn (2.35 and $5.96 \mu\text{g g}^{-1}$), and Zn (243.60 and $506.22 \mu\text{g g}^{-1}$) in *C. striatus* and *S. ferrugineus*, respectively. No comparison was made with the means of metals in the gonad of *Z. xanthurum* since only one specimen was analyzed.

4. Discussion

The concentration of heavy metals in teleost fishes may be affected by many variables most important of which are: species, body size, organ, and feeding habits (Cross et al., 1973; Eustace, 1974). The present work reveals significant differences of metal concentration among different organs of the same species, particularly the presence of lesser concentrations of metals in muscles than those in other organs examined. Similar conclusions were reported by other workers. Wahbeh and Mahasneh (1987) found lesser concentrations of metals in the muscles than those in the livers, gills, and gonads of six species of fish

from the Gulf of Aqaba. Similarly, the muscles of 21 fish species from the Red Sea proper contained lesser metal concentrations than the livers (Hanna, 1989). Comparable lower metal concentrations were also found in the muscles than the livers of Chondrichthys fishes from North Atlantic (Windom et al., 1973). In experimentally contaminated perch, the muscles contained lesser concentration of Cd than other organs (Edgren and Notter, 1980). In general, the concentrations of the metals investigated in the three species were found to be comparable with those reported from other fishes (Wright, 1976; Denton & Burdon-Jones, 1986; Miao et al., 2000; Rayment & Barry, 2000). Furthermore, the ranges of concentrations are either lower or within the ranges of the concentrations for these elements in fishes of the Red Sea (Hanna, 1989). Even the highest individual values of metal obtained for the muscle tissue in the present study are close to or within the range of those reported for many species in different environments (Table 7).

By comparison, the concentrations of metals in the present study are generally lower than those reported by Wahbeh and Mahasneh (1987) for other species from the same study area in the Gulf of Aqaba. According to Zdanowicz et al. (1992), it is uncommon to find differences in metal levels between fish species.

Table 6: Results of the analysis of variance comparing the mean concentrations of metals among the organs examined. DF, degrees of freedom; P, significance probability.

Metal	<i>Ctenocheatus striatus</i>		<i>Zebbrasoma xanthurum</i>		<i>Scarus ferrugineus</i>		Total	
	DF	P	DF	P	DF	P	DF	P
Cd	4,41	< 0.01	4, 20	0.02	4, 25	< 0.01	4,96	< 0.01
Co	4,41	< 0.01	4,17	0.01	4,22	0.02	4,90	< 0.01
Cr	4,41	0.07	4,19	0.06	4,24	0.24	4,94	< 0.01
Cu	4,41	< 0.01	4,20	< 0.01	4,25	0.04	4,96	< 0.01
Fe	4,41	0.16	4,20	0.03	4,26	0.02	4,97	0.01
Mg	4,41	< 0.01	4,20	0.06	4,26	< 0.01	4,97	< 0.01
Mn	4,41	< 0.01	4,19	< 0.01	4,26	< 0.01	4,94	< 0.01
Ni	4,41	< 0.01	4,19	< 0.01	4,24	0.01	4,94	< 0.01
Pb	4,41	< 0.01	4,19	< 0.01	4,25	0.02	4, 95	< 0.01
Zn	4,41	< 0.01	4,20	< 0.01	4,26	< 0.01	4, 97	< 0.01

Table 7: Heavy metal levels ($\mu\text{g g}^{-1}$ dry weight unless otherwise mentioned) in the muscles of fish examined from the Gulf of Aqaba, Jordan and other selected areas.

Location/Reference	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Present study (mean)	0.66	2.47	2.12	1.05	9.81	0.96	2.20	4.52	16.86
Gulf of Aqaba (1)	2.60	2.72	0.83	NM*	56.33	12.18	0.30	1.43	20.9
Red Sea (2)	0.16-3.5	0.02-0.12	0.06-4.3	1.7-39.6	NM	NM	0.20-7.0	0.05-1.3	8.4-195
Mediterranean (3)	0.33	NM	2.1	3.8	NM	NM	1.8	1.8	27.2
North Atlantic (4)	<0.1-2.1	NM	NM	1.5-3.2	NM	NM	NM	0.5-1.0	8-20
New S. Wales (5)	0.04	NM	NM	0.04-0.87	NM	NM	NM	0.45-0.67	4.24-9.56
North Carolina (6)	NM	NM	NM	0.61	NM	0.22	NM	NM	6.4
South Pacific Ocean (7)	0.01-0.46	NM	NM	0.11-2.65	NM	NM	NM	0.1-0.8	1.9-7.6
North Pacific Ocean (8)	1.2-6.3	NM	7.5-24	14-125	NM	NM	NM	9.3-31.5	84-273
Great Barrier Reef (9)	<0.1	NM	NM	0.47-2.40	NM	NM	NM	NM	4.3-41.8

* NM = Not Measured; (1) Wahbeh & Mahasneh, 1987; (2) Hanna, 1989; (3) Roth & Hornung, 1977; (4) Windom et al., 1973; (5) Bebbington et al., 1977; (6) Cross et al., 1973 ($\mu\text{g g}^{-1}$ wet weight); (7) Powell et al., 1981 (mg kg^{-1} wet weight); (8) Miao et al. 2001; (9) Denton & Burdon- Jones, 1986.

The concentration of metals in the muscle tissues were generally low and within the ranges expected for metals in muscle of fish from relatively uncontaminated locations. Moreover, the values of these metals found in the examined fish species from the Gulf of Aqaba fall below the acceptable levels for human consumption recommended by FAO (1983) and WHO (1978, 1989, 1993) which means that they do not pose a significant threat to the health of human consumers.

Site-specific differences in metal concentrations contained in specific organ may be attributed to differences in the diet of the various fish species. Wahbeh and Mahasneh (1987) reported significant differences ($P < 0.05$) in metal levels contained in the gills, muscles, livers, and gonads among six fish species from the Gulf of Aqaba. In the present work, two of the three species examined were detritus feeders and the third was an herbivorous fish feeding on algae growing on corals. There were no significant differences in metal concentrations among these species, except for Zn in the muscles, Zn, Cu, and Cd in the livers, Mg and Mn in the gills, and Cu, Mg, and Pb in the stomachs. These differences may also be partially related to feeding habits of the fish examined since the Zn, Cu, and Cd concentrations were lowest in the herbivorous fish. On the other hand, the concentrations of Mg and Mn were highest in the gills of herbivorous fish which indicates that other variables may affect the accumulation of metals, such as the rate of uptake and excretion (Cross *et al.*, 1973; Stagg *et al.*, 1982), chemical form of the metal (Bowen, 1966), and species (Murphy *et al.*, 1978). Worth mentioning that the examination of the results of the present work showed no clear trends of increasing or decreasing of metal concentrations in the fish tissue with age and size.

5. Results

The results indicate that relatively high concentrations of heavy metals were found in liver and gill of the examined species. This is in agreement with previous reports (Mears and Eisler, 1977; Eisler, 1981; Hanna, 1989; Al-Yousuf *et al.*, 2000; Calni and Atli, 2003; Karadede *et al.*, 2004). Thus, the liver and gill in fish are more often recommended as environmental indicator organs of environmental pollution than other fish organs. The results of the present study suggest the possibility of using these two organs, particularly the liver, as bioindicators of metals present in the surrounding environment. However, it is believed that monitoring of these species should be repeated on similar-sized populations on more occasions and over a longer period in order to establish if the results and associated correlations were sufficiently consistent and robust for monitoring purposes.

6. Discussion

The muscles, livers, gills, gonads, and stomachs of two detritus feeder and one herbivorous fish species collected from the Gulf of Aqaba were analyzed for nine heavy metals. The mean concentrations of heavy metals among

the organs of fish examined were generally significantly different ($P < 0.05$). Muscles contained lower metal concentrations than other organs. Comparing species, there was no significant difference in the mean concentration of metals in muscles, except Zn, which varied also among the livers of these species. Moreover, Cd and Cu means were significantly different among the livers. In gills, Mg and Mn means varied significantly. Similarly, Mg in addition to Cu and Pb varied among the stomachs of species examined. In gonads, there were significant differences between the means of Cd, Cr, Cu, Mn, and Zn in one of the detritus feeder fishes and the herbivorous fish. The concentration of metals in the muscle tissues were generally low and within the ranges expected for metals in muscle of fish from relatively uncontaminated locations. The ranges are generally lower or within the ranges of the concentrations for these elements in fishes of the Red Sea (Hanna, 1989). The values fall below the acceptable levels for human consumption recommended by FAO (1983) and WHO (1978, 1989, 1993) which means that they do not pose a significant threat to the health of human consumers. Relatively high concentrations of heavy metals were found in liver and gill of the examined species, which suggest the possibility of using these two organs, particularly the liver, as bioindicators of metals present in the surrounding environment.

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The Primary Production Conditions of Wadi Al-Arab Dam (Reservoir), Jordan

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Abstract

The purpose of this study was to investigate the effect of the primary production of phytoplankton on water quality of Wadi Al-Arab Dam Reservoir in Northern Jordan. Species successive change and composition in addition to increased rain inflow and nutrient loading seems to be the major factors behind fluctuation of total chlorophyll a (chl a) content which, was positively correlated with the phytoplankton primary production ($r = 0.63-0.68$, $P < 0.05$). The major phytoplankton species that contribute to the increase in the primary production were green algae and dinoflagellates. Below water surface, a gradual decrease in chl a concentration was observed with a lowest concentration (3.61 mg/L) being recorded at 10 m during August. Phytoplankton primary production followed a bimodal trend with two clear peaks, the first was in the wet season (March) and the second in summer (September). Total cell counts (mean count = $4040-4636 \times 10^3 \pm 1645$ cells /L) showed higher values all over the year as attributed to the trophic status of the reservoir with diatoms being the highest proportion of the total counts. Phytoplankton production was significantly correlated with dissolved oxygen concentration ($r = 0.76$, $P < 0.05$). However, surface water temperature, pH and electrical conductivity found to be negatively correlated with production ($r = -0.51$, $P < 0.05$; $r = -0.09$, $P < 0.05$ and $r = -0.24$, $P < 0.05$, respectively). Salinity and total alkalinity on the other hand, did not appear to have any particular influence on phytoplankton production.

المخلص

تهدف هذه الدراسة في البحث في أثر الإنتاجية الأولية على نوعية المياه في بحيرة سد وادي العرب. كان التغير الزمني والمكاني للعوالق النباتية بالإضافة إلى ازدياد تدفق المياه و الإثراء الغذائي لمياه البحيرة من أهم العوامل المؤثرة على تذبذب تركيز صبغة الكلوروفيل أ وبمعامل ارتباط موجب مع الإنتاجية الأولية للبحيرة ($r = 0.63-0.68$, $P > 0.05$). وليصل أقل تركيز لصبغة الكلوروفيل أ تحت سطح الماء بمقدار 3,6 مع/لتر وعلى عمق 10 متر في شهر آب. وقد لوحظ أن أهم العوالق النباتية في ارتفاع الإنتاجية الأولية للبحيرة هي الطحالب الخضراء والداينوفلاجلات. أبدت الإنتاجية الأولية للعوالق النباتية الشكل المزدوج بارتفاعين واضحين أولهما في فصل الشتاء (أذار) والثاني في الصيف (أيلول). كانت الأعداد الكلية للخلايا (متوسط العدد = $4040 - 4636 \times 10^3$ خلية/لتر) ذات قيم مرتفعة على طول السنة بسبب ارتفاع المستوى الغذائي للبحيرة واحتلال الدايتومات أعلى نسبة في العدد الكلي. ارتبطت الإنتاجية الأولية للعوالق النباتية بشكل ملحوظ مع تركيز الأكسجين المذاب ($r = 0.76$, $P < 0.05$) لكنها ارتبطت عكسياً مع درجة حرارة سطح الماء، درجة الحموضة والموصلية الكهربائية وبمعامل ارتباط سالب ($r = -0.51$, $P < 0.05$; $r = -0.09$, $P < 0.05$ و $r = -0.24$, $P < 0.05$ على التوالي). على النحو الآخر فإن الملوحة والقاعدية الكلية لم يكن لها أي أثر على الإنتاجية الأولية للعوالق النباتية.

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Keywords: Chlorophyll a; Phytoplankton; Primary Production; Wadi Al-Arab reservoir;

1. Introduction

Primary production by phytoplankton is a main process supporting life in the aquatic ecosystems and plays a prime role in their biological and chemical characteristics. Therefore, knowing the intensity and dynamics of this

process for long time scales can help to understand aquatic ecosystems as well as their water quality (Reynolds, 1984).

Reservoir water quality and productivity are to a large extent controlled by the quantity and quality of external nutrient loading. In water affected by human made effluents, such as that of Wadi Al-Arab Reservoir in Northern Jordan, high primary production resulting from excessive load of nutrients may cause problems affecting water quality for human use. Long-term changes in the

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nutrient supply to lakes represent very large-scale shifts in the balance and spectra of resources to which phytoplankton abundance and quality are known to respond (Gray, 1995).

Phytoplankton and nutrient dynamics are closely linked since nutrient uptake during algal growth is the main process that removes dissolved nutrients from the water. Algal growth is a function of temperature, light and nutrients with phosphorus, nitrogen and carbon, in addition to silicate for diatoms are the major growth limiting nutrients (Rhee, 1982).

Long-term studies of phytoplankton productivity and distribution have not been conducted in most Jordanian freshwater bodies. Therefore, the present study was aiming at investigating the biological parameters prevailing in Wadi Al-Arab Dam Reservoir and their potential effects on water quality.

2. Study Area

Wadi Al-Arab Dam Reservoir (Fig.1a) is located in the northern part of Jordan Valley, on the east bank of the Jordan Rift Valley, about 10 km south of the lake Tiberias and 25 km from Irbid City. The reservoir water comes partially from the King Abdallah Canal and partly from precipitation. The reservoir water is used to irrigate about 12,500 donums from Al Shuna to Al Baqura. It also serves as drinking water source in periods of water shortage by draining to King Abdallah Canal (Jordan Valley Authority, JVA, 1995-2002). The average annual rainfall in the Wadi Al-Arab is approximately about 400 mm (JVA, 1995-2002). Most of precipitation occurs during October to May, and months from June to September can be considered as a dry summer season. Climate of the region is considered as a Mediterranean, which is characterized by hot and dry summer, cool and wet winter. Relative humidity ranges from 49% in June to 67% in February. Frost and snowfall occur occasionally in January and February. Sunshine hour ranges from 5.0 hours in January to 11.9 hours in June (Ghrefat, 1999). Wind is light to moderate and predominantly from west to southwest. Daily evaporation causes a decrease in water level varies from 4.8 mm in January to 8.9 mm in July.

The principal features of Wadi Al-Arab Dam Reservoir are summarized as follows: the reservoir catchment's area is 262 km² with gross, effective and dead storage capacity of 20.0, 16.9 and 3.1 million cubic meters, respectively. The estimated precipitation per year is 7000 m³ and the annual total discharge is 33 million m³ (JVA, 1995-2002). The reservoir live storage capacity during the study period (February 2001 to February 2002) maximized in April, 2001 to 13182000 m³ and the minimized in November, 2001 to 1360000 m³.

3. Materials and Methods

3.1. Sampling

Two sampling stations (SI and SII) were selected for investigation. Station I was closer to the shore of the lake

and station II represents the open area and is located in the middle of the reservoir about 350 m from station I, at a fixed point by a flag planted there where the boat usually reach water (Fig.1.b). Water samples in two plastic bottles of 1 liter volume were carried out monthly from each station during the study period. Primary production was measured at the tow stations and samples for chlorophyll *a* measurements were obtained from a depth of 30 cm below the water surface in each station.

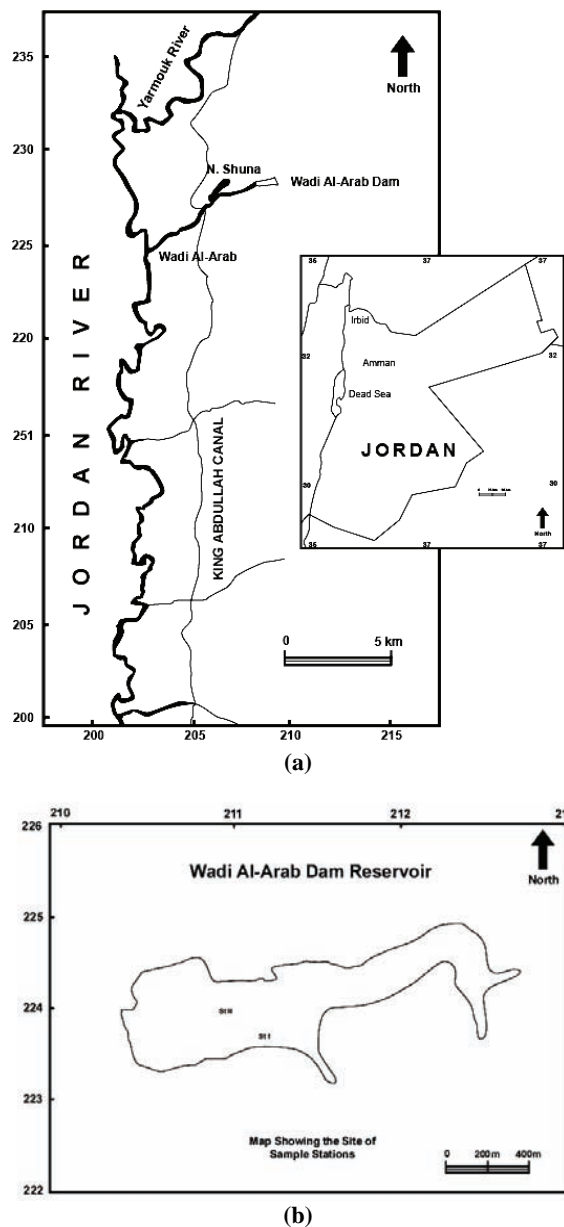


Figure 1. a. Location map of the study area; b. Map showing the locations of sample stations in Wadi Al-Arab dam reservoir.

3.2. Biological analysis

3.2.1. Cell count

3.2.1.1. Lackey drop (Microtransect) counting method

One-liter samples were collected from each station and fixed with 5 ml of 40% formaline. Upon return to the lab, samples were transferred to a 1L cylinder and left to settle for 72 hours. This period was found to be enough for all algal cells to settle down. Later, 90 % of the supernatant water was decanted, leaving about 100 ml of phytoplankton concentrate. Cells were counted according to APHA (1998). In brief, the concentrated sample was shaken vigorously then 0.1 ml of the suspension was placed on a blank microscopic slide and covered with a 22 x 22 mm glass cover slip. Organisms were counted in four strips all over the width of the cover slip. Number of cells/ml was calculated according to:

$$\text{Cells / ml} = \frac{C \times A_t}{A_s \times S \times V}$$

C : total number of organisms counted in the counted strips.

A_t : area of the cover slip, mm

A_s : area of one strip = Number of fields counted × area of one field.

S : No. of strips counted, 4.

V : volume of sample under the cover slip, ml = 0.1ml

3.2.1.2. Chlorophyll *a* and phaeopigment analysis

Samples for chlorophyll *a* and phaeopigments analysis were thoroughly shaken, filtered through GF/C glass fiber filters (25 mm diameter) using Millipore filtration unit and vacuum pump at low pressure. The glass fiber filter was grinded with few drops of 90 % acetone then transferred to glass tubes in which acetone was added to a volume of 15 ml. The filtrate was left over night and was centrifuged at 2500-3000 (rpm) for 10 minutes. Finally the supernatant was measured spectrophotometrically at 665 nm and 750 nm wavelengths (Milton Roy, USA) before and after acidification with 0.1N HCL as described in APHA (1998). The concentration of chlorophyll *a* and other phaeopigments were calculated according to Vollenweider (1974).

3.2.1.3. Primary production measurement: Light / Dark bottles method

Phytoplankton primary production was measured by the oxygen light and dark bottle technique (APHA 1998). Winkler method was followed for the estimation of dissolved oxygen in the incubation bottles.

A measurement of net photosynthesis (photosynthesis in excess of respiration) can be obtained by measuring the gain in oxygen concentration in the transparent bottle. Net photosynthesis is equated with net oxygen evolved and is obtained by subtracting the oxygen content of the water before incubation from oxygen content of the transparent bottle following incubation. A measurement of gross photosynthesis can be obtained by adding the amount of respiratory oxygen to the net oxygen evolved. Respiratory oxygen is calculated by subtracting the oxygen content of the dark bottle after incubation from the oxygen content of

water before incubation. With suitable calculations of both net and gross photosynthesis, primary production can be expressed in units of assimilated carbon per unit of time.

In the present study, 6 Winkler bottles (BOD bottles) of 300 ml volume were used in each station. Four bottles were left clear and two bottles were painted black and wrapped with aluminum foil. Oxygen concentration in one of the clear bottles was determined immediately at the beginning of the experiment and considered as the initial oxygen concentration. The remaining bottles were incubated in situ for 3 hours. After the incubation period, oxygen concentration was determined in all bottles as the following and average values for light and dark bottles were recorded

3.3. Primary production calculation

Gross primary production (mg O₂ / L) was measured by subtracting the concentration of dissolved oxygen (DO) in the dark bottle from that in the transparent bottle. However, net primary production was measured by subtracting (DO) in the initial bottle from that in the transparent bottle. To convert (mg O₂ / L) into (mg C / m³ / hr) the following formula was used:

$$(\text{mg C} \setminus \text{m}^3 \setminus \text{hr}) = \frac{\text{mg O}_2 / \text{m}^3 / \text{hr} \times (12/32) \times 1000}{\text{time of incubation}}$$

12/32: factor to convert oxygen to carbon; 1000: to convert ml to m³.

3.4. Physical and chemical analysis

3.4.1. Physical and chemical parameters

Water temperature, pH, salinity and conductivity were measured in situ upon sampling using a multiparameter portable instrument (WTW, Multiline F / SET-3, Germany).

3.4.2. Dissolved oxygen (DO)

Dissolved oxygen concentration in the incubation bottles was measured following Winkler method as described in APHA (1998).

4. Statistical analysis

Analyses of variance for all data were performed using statistical analysis system (SAS Institute Inc., 2000). Means were separated by the least significant differences (LSD) at α = 0.05.

5. Results and Discussion

Fluctuation of water level at Wadi Al-Arab reservoir is a combined result of an irregular variation of inflow and outflow. The main source of water to the reservoir is precipitation, which is confined to short period in winter. On the other hand, water outflow from the reservoir increased during summer owing to an increasing demand of water for irrigation and drinking. It is estimated that about 65,000 m³ of water is pumped daily from the reservoir for agricultural uses in Al-Shuna and nearby

villages (JVA, 2000). A wide fluctuation in water level, however, is a common feature in reservoirs and has some effect on their ecology through, for example, an enhanced nutrient exchange between pelagic and littoral zones of the reservoir (Andrew and Pfiester, 1995).

Total cell counts showed higher values all over the year with diatoms being the highest proportion of the total counts. The mean cell count through the study period was $4040 \times 10^3 \pm 1410$ cell /L at station I and $4636 \times 10^3 \pm 1645$ cell /L at station II (Fig. 2a). Maximum cell count recorded at station I was 7103×10^3 cell in December and the minimum was 1857×10^3 cell /L in July. At station II, the maximum cell count recorded was 8810×10^3 cell /L in December and the minimum was 1606×10^3 cell /L in February 2002.

Changes in cell counts provide good indication of phytoplankton successional pattern. Taxonomical uncertainty, however, may limit the full understanding of successional changes (Hart, 1996). The irregularity in cell count changes in the reservoir can not be related to any particular environmental parameter. It is most likely to be a combined effect of a transmissible distribution (Fogg and Thake, 1987) and succession interruption. Succession interruption, however, is a common feature of warm reservoirs which caused by changes in the depth of mixed layer (Lewis, 1996). Growth strategies on the other hand play a major role in the observed variation of species peaks.

Nutrient concentrations have not been measured in this study, but it was shown earlier (Saadoun et al., unpublished data) that Wadi Al-Arab reservoir is eutrophic with high nutrients loads all of the year round. Therefore, it is unlikely that nutrients in the reservoir are limiting factors for phytoplankton cell densities. It is most likely that temperature, light limitation and physiological status of species play a more influencing role in species succession. Furthermore, the absence of a detailed study on the phytoplankton of Wadi Al-Arab reservoir makes it difficult to extract comparison of species occurrence and distribution both within and between years.

Species composition seems to be the major factor behind fluctuation of total chlorophyll *a* content in the reservoir. In March 2001, chl *a* concentration was 29.38 mg/L where phytoplankton was mostly dominated by the dinoflagellate *Peridinium sp.* Phytoplankton species composition had changed remarkably in the next month (April) where the chlorophytes *Chlorella sp.* and *Chlamydomonas sp.* became more common. This successive change of species was accompanied with a rapid increase in chl *a* concentration reaching 107.79 mg/L. Similar conditions, however, have also been noted in other reservoirs (Reynolds, 1984). From May to the end of the year, fluctuations in concentration of chl *a* were rather low with its minimum in November when diatoms dominate the population.

Significant and abrupt increase in chl *a* concentration was observed in February 2002 at both stations. Chlorophyll *a* concentrations were rather high in Wadi Al-Arab reservoir with a seasonal change that was related to water inflow to the reservoir in the wet season (September to February) as compared to a marked decrease in summer months (May to August) (Fig. 2b). Chl *a* mean concentrations over the study period were (39.62 mg / m^3)

and (46.34 mg / m^3) at station I and II, respectively, with the highest level (120.42 mg / m^3 and 107.97 mg / m^3) recorded in February, 2002, at station I and in April, 2001 at station II, respectively. However, the lowest chl *a* concentration recorded at station I was 15.83 mg / m^3 in August where cell densities were rather low resulting in a relatively more transparent water. At station II, the minimum concentration recorded was 19.56 mg / m^3 in November. In February 2002, elevated level of chl *a* was correlated with the increased rain inflow, nutrient loading and species distribution. In general, the factors which are known to decrease chl *a* in lakes and reservoirs such as light availability (Desortova, 1981; Hunter and Laws, 1981; Kotut et al. 1999) and algal size (Malone, 1980) seem to be of less importance in highly eutrophic water bodies like Wadi Al-Arab reservoir.

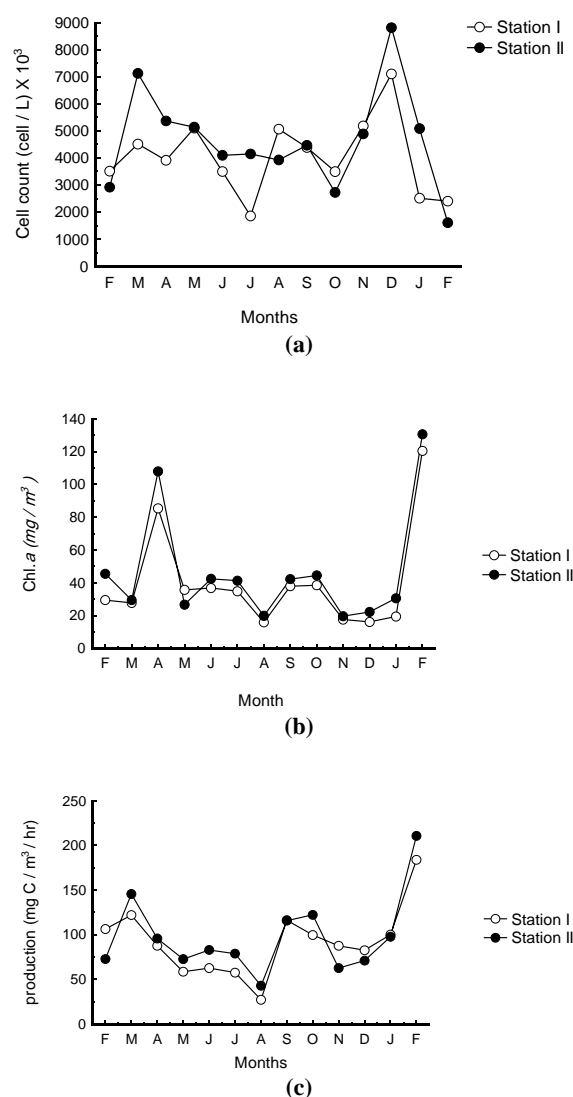


Figure 2. Total cell count (cell/L) (a), chlorophyll *a* (mg/m^3) concentration (b) and phytoplankton primary production ($\text{mg C/m}^3/\text{hr}$) (c) at stations I and II.

Chl *a* concentration was determined at the water surface and at 1, 5 and 10 m below the water surface for the period April to August. A gradual decrease in chl *a*

concentration with depth was observed with the highest values always at the surface (Fig. 3). The lowest concentration recorded (3.61 mg/L) was at 10 m below the water surface and during August (Fig. 3e). Depletion of chl *a* values with depth was observed all the year round and may be attributed to the low cell densities under water surface.

Results of phaeopigment concentration (data not shown) revealed no seasonal pattern. The maximum concentration (39.57 mg / m³) recorded was in May at station I, while at stationII, the maximum concentration (18.84 mg/m³) was recorded in April. The lowest concentration (1.1 mg/m³) was recorded in September at station II and (0.52 mg/m³) in October at station I. Phaeopigments which reflect concentration of non-functional chl *a* were rather low in surface water of the reservoir with minor fluctuations throughout the period of study. Increased levels of phaeopigments were observed below the water surface during May-July period, which is attributed to the rapid sinking of dead phytoplankton cells.

Phytoplankton primary production of the reservoir showed a seasonal pattern with an increase in the wet season and a decrease in summer. The mean primary production at station II was higher than that at station I with values of 97.72 mg C m⁻³hr⁻¹ and 91.60 mg C m⁻³hr⁻¹ at station II and I, respectively. Phytoplankton primary production was generally high and followed a bimodal trend with two clear peaks, the first was in the wet season (March) and the second in summer (September) at both stations. In February 2002, elevated levels of primary production were recorded with 210.55 mg C m⁻³hr⁻¹ at station II and 183.75 mg C m⁻³hr⁻¹ at station I. The maximum production recorded in February 2002 at both stations, coincided with the marked increase in nutrient loading to the reservoir. However, a considerable decrease in the primary production rate was observed in Summer with a minimum of 27.08 mg C m⁻³hr⁻¹ at station I. The

lowest production recorded at station II was 42.90 mg C m⁻³hr⁻¹ in August (Fig. 3e). The minimum productivity of the reservoir was in August at both stations, which is correlated with a lower chl *a* concentration.

The major phytoplankton groups that contributed to winter rise of primary production were green algae and dinoflagellates (Saadoun et al. 2008). A positive correlation was found between chl *a* and primary production at both stations ($r = 0.66$, $P < 0.05$). In discussing the correlation of different parameters with primary production, chl *a* appears to be the closest factor in this regard and is widely used as an indirect measure of phytoplankton productivity (Voros and Padisak, 1991). Such close relation results from the fact that chl *a* is the most abundant pigment in plant living material. Chl *a*, on the other hand, was found to decline with reduced nutrients levels (Hunter and Laws, 1981), but such condition was not found in the reservoir since nutrient were above normal concentration all of the time. Therefore, a strong correlation between primary production and chl *a* concentration in Wadi Al-Arab reservoir is predictable. Minimum chl *a* concentration at station I in August 2001 coincided with the lowest production of the reservoir, also when chl *a* peaked in February 2002, it was accompanied with a peak of phytoplankton production. Positive correlation was evident between chl *a* and primary production at the two stations ($r_1 = 0.63$, $r_2 = 0.68$, $P < 0.05$, respectively).

Many factors are well known to affect the primary production of the phytoplankton including nutrients loading, species composition, light and temperature. The data of water temperature, pH, salinity, dissolved oxygen and conductivity are not shown (Saadoun et al. unpublished data). The relation between temperature and primary production showed a degree of correlation in terms of seasonality as primary production increased in winter months and decreased in summer months.

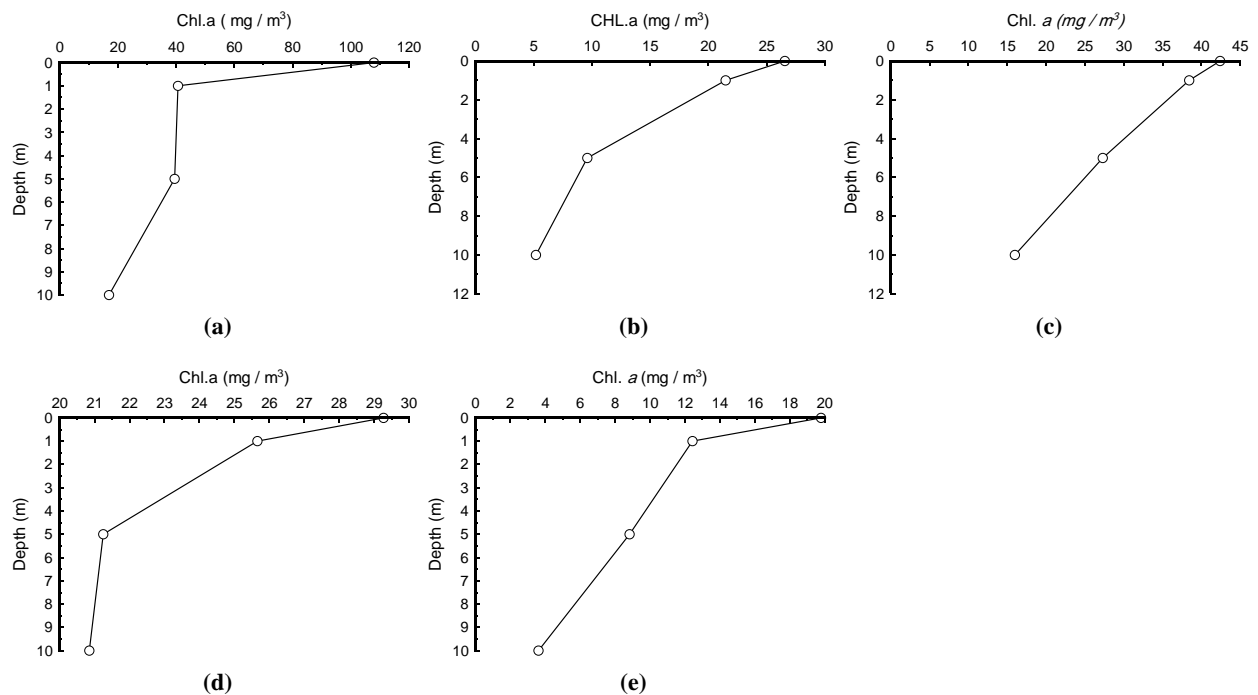


Figure 3. Vertical profile of chlorophyll *a* (a: April, b: May, c: June, d: July and e: August) at station II.

However, negative correlation ($r = -0.51$, $P < 0.05$) was recorded between primary production and surface water temperature. This, however, can be referred to the photoinhibition as light intensity is considerably high during summer in this part of the world. Photoinhibition is known to occur in *in situ* incubated bottles in shallow waters during warm seasons (Coldman and Dennett 1984). As primary production estimation depends mainly on the rate of photosynthesis reaction, and as the pH of the water correlated with CO₂ consumption by phytoplankton population, one can relate the pH of the water to its productivity. But in the reservoir, values of pH showed no seasonality with a mean of 7.92 at the two stations and was negatively correlated with productivity ($r = -0.09$, $P < 0.05$) (Saadoun et al., unpublished data).

Also, the electrical conductivity of the reservoir showed high values without any pattern of variation. This stability of the reservoir salts levels reduce its influence on the productivity of the phytoplankton, so it was found to be negatively correlated with production ($r = -0.24$, $P > 0.05$). Variation of total alkalinity in the reservoir is similar to that of electrical conductivity, the two parameters were highly correlated ($r = 0.92$) and have no obvious influence on phytoplankton production. Salinity, on the other hand, did not appear to have any particular influence on production (Saadoun et al. unpublished data).

6. Conclusion

Primary production in Wadi Al-Arab Dam reservoir during the study period was generally high and showed pattern of seasonally characterized by increase in wet season and a decrease in summer months. It was significantly correlated to dissolved oxygen concentration, but, negatively correlated with the surface water temperature and pH. As chlorophyll *a* is the most abundant pigment in plant living material, chlorophyll *a* appears to be the closest factor to be correlated to primary production. Positive correlation was evident between chlorophyll *a* and primary production at both stations.

Acknowledgement

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Virtual Reference Values for STR Genetic Loci Assignment in Forensic Arenas: A Jordanian-Based Study

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Abstract

Short tandem repeat (STR) genetic loci were assigned for use in forensic investigation for the Jordanian population by using a computer program. The program comprised two virtual reference values, the maximum virtual target power of discrimination (VTP_D)_{max} and the maximum virtual observed heterozygosity (VH_{obs})_{max}. These two virtual reference values prompted the selection of the TH01, FES/FPS, D18S51, and Penta D STR genetic loci from 17 STR genetic loci used worldwide in forensic applications. The four STR genetic loci showed a combined power of discrimination of 0.999987, indicating that these four STR genetic loci are forensically viable for the Jordanian population. This method of utilizing a computer program to assign genetic loci would reduce the time and cost of DNA typing.

المخلص

لقد تم وضع برنامج محوسب لتحديد أفضل المواقع الجينية الجنائية (STR) لأستخدامها في المجتمع الأردني. يضم البرنامج المحوسب قيمتين افتراضيتين مرجعيتين لتحديد أفضل المواقع الجينية الجنائية. هاتان القيمتين تدعوان القيمة الافتراضية القصوى للتمييز بين الأفراد (VTP_D)_{max} والقيمة الافتراضية القصوى لتعددية الأليلات التابعة للمواقع الجينية الجنائية (VH_{obs})_{max}. لقد حفزت هذه القيم الافتراضية اختيار أربعة مواقع جينية جنائية هي: TH01, FES/FPS, D18S51, Penta D من بين سبعة عشر موقعا جينيا جنائيا مستخدمة في أنحاء العالم. لقد أظهرت المواقع الجينية الأربعة قدرة كبيرة على التمييز بين الأفراد في المجتمع الأردني تعادل 0.999987. أن أستخدام مثل هذا البرنامج المحوسب يساهم في خفض تكلفة وتقليل الوقت المستغرق في تحليل المادة الوراثية DNA.

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Keywords: Forensic Science, Genetic Loci, Short Tandem Repeat, Virtual Reference Values;

1. Introduction

The advent of short tandem repeats (STRs) into forensic arenas has revolutionized criminal and legal investigations. The criminal and justice authorities worldwide have utilized the ability of such STRs to solve questionable settings including paternity disputes and human individualization and identification (Budowle et al., 2001; Butler JM, 2006).

Several parameters were established and adopted for the selection of the STR loci as genetic markers in forensic analysis. Such parameters include STR's sequence length (Weber and May, 1989), independent inheritance (Tracey, 2001), polymorphic nature (Weber and May, 1989), heterozygosity which is defined as the probability that two alleles drawn from a population are not identical and amenability to amplification by the polymerase chain reaction (Yamamoto et al., 1997; Lazaruk et al., 1998; Wenz et al., 1998). The polymorphic nature of each STR

genetic locus which is a reflection of the STR's allelic window (number of alleles per STR genetic locus) has characterized these short sequences with a highly discriminating power that is useful in human individualization and identification. The discriminating power or power of discriminating (P_D) is defined as the probability that two individuals chosen at random will possess different genotypes for the marker being tested. However, studies have shown demonstrable variation in the allelic windows and also a certain degree of significant differences in the allelic frequency distributions for some of the forensic STR genetic loci among different human populations (Edwards et al., 1992; Gamero et al., 2000; Hamad et al., 2001; Hayes et al., 1995; Lins et al., 1998; Salem et al., 2003; Yasin, 2002). Since the allelic frequencies distribution of the allelic window for each STR genetic locus is implemented in the determination of P_D , it is expected that the variation in the allelic frequencies distribution is also exhibited to a certain degree in their discriminatory power. Thus, discrepancies in the values of P_D of STR genetic loci among human

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populations are amenable. Such discrepancies are arguable since they depend on the allelic frequencies distribution in human populations. However, they might still shed some doubt on the efficacy of implementation of the same set of STR genetic loci in the forensic arenas for all human populations. Therefore, the legal implications of such discrepancies should be considered; and prompt to search for a tangible method for determining the efficacy of implementation of a certain genetic locus in a human population.

In this study, the most applicable STRs for forensic investigations in the Jordanian population were selected by developing a computer program. The program utilized two computer-based virtual reference values that depend on the allelic window of each STR genetic locus: the maximum virtual target power of discrimination $(VTP_D)_{max}$ and the maximum virtual observed heterozygosity $(VH_{Obs})_{max}$, i.e., the maximum possible values that could exist in a Hardy-Weinberg population.

2. Materials and Methods

The STR genetic loci raw data implemented in this study were described earlier (Hamad et al., 2001; Salem et al., 2003; Yasin et al., 2002). The data comprised the genotypes and allelic frequency distributions of seventeen STR genetic loci (Table 1).

Figure 1 shows a snapshot of the main menu of the developed software used in this study. The input to program includes various parameters such as population name, population size, sample size, and the experimental STR loci. The developed software generates the virtually observed genotypes of the experimental STR genetic loci within the sample individuals. The results obtained for each STR genetic locus include the allelic frequency distributions, the observed and expected genotypic frequency distributions, and the resultant real and virtual

forensic parameters values for all of the experimental STR genetic loci.

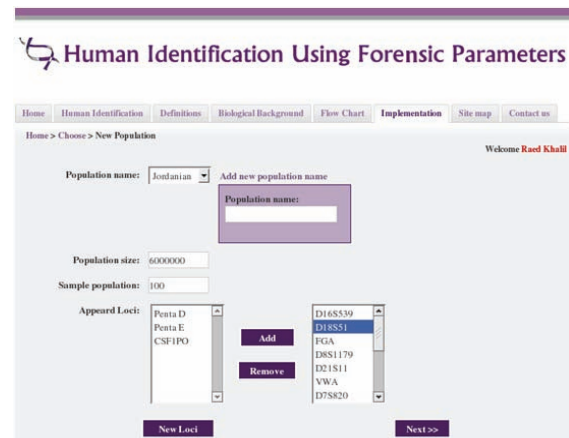


Figure 1: A snapshot of the main menu of the developed software used in this study.

The software comprised two computer-generated virtual reference values, the $(VTP_D)_{max}$ and the $(VH_{Obs})_{max}$. Both reference values require the generation of all virtual genotypes for each genetic locus according to the formula $n(n+1)/2$, where n represents the number of alleles assigned per an STR genetic locus. The $(VTP_D)_{max}$ assumes that in any human population all alleles comprising the allelic window for any STR genetic locus are present in the population. Therefore, the number of all genotypes that could possibly exist or emerge in the population can be determined and listed.

The computer software would randomly generate observed numbers for each genotype; and then, determine the allelic frequencies distribution for each allele in the STR allelic window.

Table 1: Virtual Reference Values for the Seventeen STR Genetic Loci.

STR Locus	Power of Discrimination (P_D)			Observed Heterozygosity (H_{Obs})		
	Actual P_D	$(VTP_D)_{max}$	p value	Actual H_{Obs}	$(VH_{Obs})_{max}$	p value
TH01	0.94360	0.98437	0.151	0.75	0.833	0.0751
TPOX	0.8284	0.98138	0.0003*	0.45	0.81	0.0000*
vWA	0.90800	0.98845	0.0139*	0.84	0.856	0.3765
Penta D	0.95700	0.99360	0.097	0.87	0.886	0.3650
Penta E	0.97640	0.99583	0.2417	0.73	0.928	0.0001*
CSF1PO	0.85320	0.98670	0.0006*	0.85320	0.98670	0.0006*
FGA	0.95300	0.99833	0.039*	0.84	0.949	0.0065*
FES/FPS	0.87640	0.95213	0.0572	0.74	0.713	0.3344
F13A1	0.91660	0.98848	0.0178*	0.78	0.864	0.0610
D3S1358	0.89240	0.98664	0.0057*	0.84	0.851	0.4149
D5S818	0.89860	0.98135	0.0146*	0.72	0.815	0.0567
D7S820	0.92120	0.98437	0.0365*	0.80	0.852	0.1666
D8S1179	0.93480	0.99416	0.0244*	0.76	0.898	0.0051*
D13S317	0.89200	0.98131	0.0102*	0.79	0.822	0.2839
D16S539	0.88260	0.98137	0.0061*	0.82	0.824	0.4706
D18S51	0.96180	0.99727	0.078	0.88	0.934	0.0951
D21S11	0.95040	0.99726	0.0392*	0.80	0.938	0.0021*

*: Significant level of difference

The sample size was virtually assigned at 1000 virtual individuals. These allelic frequencies are used to calculate the P_D at each random generation of observed number for each genotype of an STR genetic locus. The computer software would then determine the $(VTP_D)_{max}$ reference value that could result. This $(VTP_D)_{max}$ reference value is used as a reference target value to determine the applicability of an STR genetic locus in forensic arenas in the Jordanian population. The raw data of the power of discrimination based upon the allelic frequency distributions present in the Jordanian population for each STR genetic locus were compared with the $(VTP_D)_{max}$ reference value. Based on all virtual genotypes, the software determined the $(VH_{Obs})_{max}$ reference values for all of the seventeen STR genetic loci. The $(VTP_D)_{max}$ and the $(VH_{Obs})_{max}$ reference values for the seventeen STR genetic loci were considered to be the target values for determining the applicability of any genetic STR locus in human identification settings in Jordan.

Statistical analysis between the raw data and the virtual data for all the seventeen STR genetic loci was carried out using the STATISTICA software for Windows, 1995 version (StatSoft, Tulsa, OK, USA), where the test for difference between two percentages computes the significance level for the difference between two percentages; both one-sided and two sided tests. The p -level was computed based on the t -value for the respective comparison according to the following formulas:

$$|t| = \sqrt{[(N1+N2)/(N1+N2)]x[P1-P2] / \sqrt{Pxq}}$$

Where,

$$P = (P1xN1 + P2xN2) / (N1 + N2)$$

$$q = 1 - P$$

The degrees of freedom are computed as:

$$N1 + N2 - 2$$

3. Results

In view of the computer-generated $(VTP_D)_{max}$ values (Table 1), the computer software was able to randomly generate the virtual genotypes and allelic frequencies for all of the STR genetic loci. The allelic frequencies were used to generate the $(VTP_D)_{max}$ values for each genetic STR locus; while the $(VH_{Obs})_{max}$ values were calculated using the computer-generated virtual genotypes.

The $(VTP_D)_{max}$ values varied from 0.99833 for the FGA genetic locus to 0.95213 for the FES/FPS genetic locus. These indicated that all of the seventeen genetic STR loci possess high virtual target discriminatory power that can be applied in determining the efficacy of each respective genetic locus for forensic applications. Furthermore, the results showed that the $(VH_{Obs})_{max}$ values for the seventeen STR genetic loci varied from 0.949 for the FGA STR genetic locus to 0.713 for the FES/FPS STR genetic locus.

In order to evaluate the efficacy of forensic implementation of each of the seventeen STR genetic loci in the Jordanian population, the previously determined

allelic and genotypic windows for the Jordanian population were used to recalculate the various forensic parameters. They include the actual P_D and H_{Obs} values for each STR genetic locus. These parameters were compared with previously published data, particularly, with their respective $(VTP_D)_{max}$ and $(VH_{Obs})_{max}$ values for each STR genetic locus (Table 1).

Data analysis showed that all previously published forensic parameters are similar to those generated by the computer software (Hamad et al., 2001; Yasin, 2002; Salem et al., 2003). In this work, attention was paid for the actual P_D and H_{Obs} values for all of the seventeen STR genetic loci. The actual P_D values varied from 0.97640 for the Penta E genetic locus to 0.82840 for the TPOX genetic locus. The actual H_{Obs} values varied from 0.88 for the D18S51 STR genetic locus to 0.45 for the TPOX STR genetic locus.

Statistical comparison of the $(VTP_D)_{max}$ values with the actual P_D values showed significant differences ($p < 0.05$) at twelve genetic STR loci, namely, TPOX, vWA, CSF1PO, FGA, F13A1, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, and D21S11 (Table 1). Absence of significant differences was demonstrated for the TH01, Penta D, Penta E, FES/FPS, and D18S51 STR genetic loci ($p > 0.05$). Furthermore, comparison between the $(VH_{Obs})_{max}$ values and the actual H_{Obs} values (Table 1) showed statistically significant differences ($p < 0.05$) at six STR genetic loci, namely, TPOX, Penta E, CSF1PO, FGA, D8S1179, and D21S11. No significant differences ($p > 0.05$) were shown for the TH01, vWA, Penta D, FES/FPS, F13A1, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, and D18S51 STR genetic loci data.

4. Discussion

Table 1 indicates that the actual P_D values for the seventeen genetic loci are high enough to enable them to show a high discriminatory power among Jordanian individuals. Nevertheless, twelve of these STR genetic loci could be recommended for exclusion from the STR genetic loci panel used for forensic investigations in the Jordanian population because there was a significant difference between the virtual and actual P_D values for these loci. Such exclusion reduces the number of STR genetic loci being used to a panel of five loci that includes the TH01, Penta D, Penta E, FES/FPS, and D18S51. The combined power of discrimination for these five genetic loci was calculated at 0.999999692 (Combined $P_1 = 0.000000308$). Thus, combining these five STR genetic loci into a single panel should yield satisfactory levels of population resolution and individual identification in Jordanians considering the small size of the Jordanian population that approximates 5.65 million according to the latest Jordanian census bureau data (<http://www.dos.gov.jo>).

The presence of significant level of differences between the $(VH_{Obs})_{max}$ values and the actual H_{Obs} values of the TPOX, Penta E, CSF1PO, FGA, D8S1179, and D21S11 STR genetic loci indicated that the exhibited level of observed heterozygosity for these six STR genetic loci in the Jordanian population is theoretically not acknowledgeable. Accordingly, these STR genetic loci can be excluded from any forensic STR set used for human

identification or individualization in the Jordanian population. Furthermore, the data demonstrated that the TH01, vWA, Penta D, FES/FPS, F13A1, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, and D18S51 STR genetic loci exhibit high heterozygosity levels, hence high genetic variability in the Jordanian population. This indicates their applicability in forensic investigation in Jordan. These eleven STR genetic loci show an exceptionally high combined P_D of 0.9999999999891 (Combined $P_1 = 1.0914 \times 10^{-12}$).

Combining both of the virtual reference values of $(VTP_D)_{\max}$ and $(VH_{\text{Obs}})_{\max}$ can greatly enhance the selection of the forensic STR genetic loci panel used for human identification and individualization in a population. In Jordanian population, the forensic STR genetic loci panel could comprise four STR genetic loci, namely, the TH01, Penta D, FES/FPS, and D18S51. The combined Power of Discrimination for these four loci was calculated at 0.999987 (Combined $P_1 = 0.000013$). This is a relatively high discriminatory power considering the population size of Jordan.

In this work, we established a virtual method that is able to determine the fitness of and assign STR genetic loci into a low-cost, time-saving, and a highly discriminatory forensic STR panel. However, it remains for the national forensic authorities in the world to evaluate the applicability of any forensic genetic loci in their respective populations taking in consideration the population size and possible exchanges with neighbouring countries. It is possible that a higher population size or mosaic population could require increasing the number of STR genetic loci to achieve significantly higher discriminatory power.

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Distribution of Diazinon in Water, Sediment and Fish from Warri River, Niger Delta Nigeria

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Abstract

This paper presents the first attempt to quantify the levels and distribution pattern of diazinon in surface water, sediment and fish (*Chrysichthys furcatus* and *Tilapia zilli*). The samples were collected from three stations (Ovwian, Ekakpamre and Ovu) of Warri River in the western Niger Delta of Nigeria in 2006 during the dry (January-April) and wet seasons (May – August). A total of 96 samples made up of 24 samples each for water, sediment and fish were analyzed in this study. The pesticide levels were analyzed using high performance liquid chromatography (HPLC model CECIL 1010) to elucidate its distribution in various environmental compartments. The concentrations of the pesticide in the matrices ranged from; 0.01-3.61 µg/L (water), 0.01-3.64 µg/gdw (sediment), 0.01-7.51 µg/gdw (*C. furcatus*) and 0.01-1.13 µg/gdw (*T. zilli*). From this result, decreasing order of occurrence of the pesticide is as follow; fish > sediment > water. The concentrations observed in fish (*C. furcatus*) were higher than the levels observed in sediment and water suggesting bioaccumulation of the pesticide by the fish. Spatial variations occurred with downstream stations having statistically higher concentrations in all matrices at $P < 0.05$. Seasonal variations occurred with higher concentrations in dry season for water and sediment only, while the fish species had higher concentrations in the wet season. The observed values were above the ecological bench marks (0.02 µg/L) recommended by Nigeria Environmental Protection Agency and European Union. They were also relatively higher than previous studies on the Nigerian environment, an observation that calls for regular monitoring of the Niger Delta water bodies.

المخلص

تتناول هذه الورقة أولى المحاولات لقياس مستويات و أنماط توزيع الديازينون في المياه السطحية و التربة و الأسماك كريسختس فيركاس و تلابيا زيلي . جمعت العينات من ثلاث محطات (أوفيان و ايككابامير و أفو) من نهر واري في دلتا النيجر الغربية خلال موسم الجفاف عام ٢٠٠٦ (كانون الثاني – نيسان) و خلال الموسم الرطب (أيار – آب) . تم جمع ٩٦ عينة تتكون من ٢٤ عينة من الماء و التربة و نوعي السمك . تم تحليل مستوى المبيد الحشري بواسطة الكروماتوغرافيا عالي الجودة . بلغت تراكيز المبيد من ٠.١ – ٣,٦٤ مايكروغرام / اللتر في الماء ، و ٠.١ – ٣,٦٤ مايكروغرام / غرام جاف في التربة ، و ٠.١ – ٧,٥١ مايكروغرام / غرام جاف في سمكة كريسختس ، و ٠.١ – ١,١٣ مايكروغرام / غرام جاف لسمكة التلابيا ، لذا يمكن ترتيب التركيز من العالي إلى المنخفض : سمك < التربة < الماء . يدل التركيز العالي في السمكة على التراكم الحيوي للمبيد في السمك . أما التوزيع المكاني للمبيد فكان التركيز عالياً في أسفل النهر ، أما التوزيع الزمني فكان التركيز في الموسم الجاف أعلى منه في الموسم الرطب في الماء و التربة و على العكس في السمك . كانت التراكيز أعلى من التراكيز التي حددتها وكالة البيئة النيجيرية و الاتحاد الأوروبي (٠.١ مايكروغرام / لتر) و هذا يستدعي مراقبة مياه دلتا النيجر .

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Keywords: *C. furcatus*; Diazinon; Niger Delta; Sediment; Surface water; *T.zilli*; Warri River;

1. Introduction

Pesticides play an important role in agriculture and in pest control worldwide. In order to feed the ever - increasing human population in Nigeria, it has become

necessary to use pesticides. Pesticides usage in Nigeria dates back to 1948. By 1974, 21 types of organophosphates and carbamate insecticides had been introduced into Nigeria. There was a phenomenal increase in this number in 1979, when the Federal Government introduced the Green Revolution Programme (Badejo and Sosan, 2005).

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Insecticides play an important role in aquatic ecosystems as documented by the accumulated data on their detrimental effects to community structure, reproduction, and developmental process among several taxa including macro invertebrates, amphibians, birds, and other wildlife (Colborn et al., 1999; Thompson et al., 1996)

Diazinon is an organophosphate insecticide; common trade names include Spectracide, Basudin™, Knoxout™. Globally the pesticide is the fifth most commonly used pesticide used by homeowners, with two to four million pounds used annually (Cox Caroline, 1992). It originated with Ciba-Geigy in 1952. Various diazinon formulations are widely used in agriculture and for structural pest control, besides being used on lawn, and home gardens. Important target pests for diazinon applications include cockroaches, fleas, ticks, aphids, scales, mites, ants, crickets, flies, and grubs (Extension Toxicology Network, 1996). Diazinon may be found in formulations with a variety of other pesticides. Depending on form, the EPA has classified diazinon as a toxicity class II or III pesticide, based on a scale of I to IV, I being the most toxic class (Meister, 2000).

Diazinon is a moderately acutely toxic broad-spectrum insecticide, with a LD₅₀ of 350 to 400 mg/kg for humans (Doherty et al., 1982). Like other organophosphate pesticides, diazinon affects the nervous system through the inhibition of AchE, an enzyme needed for proper nervous system function. Diazinon is easily absorbed through the skin, and can be synergistic with other chemicals (meaning that the two together are more toxic than either alone), including pyrethrins and certain chemicals used in pharmaceuticals (Plott, 1988). The pesticide is highly toxic to fish, aquatic invertebrate, predatory or parasitic insects, soil microbes, and mites. (Coupe et al., 2000).

The United State Geological survey data show that "diazinon is the most commonly found insecticide in surface water nationally" (Hoffman et al., 1994). Diazinon was one of the five pesticides found to be able to concentrate in fog droplets. In 1995, the U.S. Geological survey began a study to determine the occurrence and temporal distribution of 49 pesticides and pesticide metabolites in air and rain samples from an urban and an agricultural sampling site in Mississippi. The study found that every rain and air sample collected from the urban and agricultural sites had detectable levels of multiple pesticides. Diazinon was found to have the highest concentration at the both sites (Glottfelty et al., 1987)

There is currently widespread concern about the concentration and effects of chemical pollutants such as diazinon on aquatic resources in the western Niger Delta (Egborge, 2001; Ezemonye, 2005). This is not unconnected with indiscriminate use of pesticides to control water-inhabiting pests and disease vectors in the region (Badejo and Sosan, 2005)

Warri River, a major navigable channel of the Niger Delta, southern Nigeria. It takes its origin from around Utagba Uno and flows through zones of freshwater swamps, mangrove swamps, and coastal sand ridges. The

river is a relatively large water body which stretches within latitudes 5°21' N - 6°00' N and longitude 5°24' - 6°21' E, covering a surface area of about 255 sq. km with a length of about 150 km (Tetsola, 1980; Gabriel, 1986; Netherlands Engineering Consultants, 1954). It drains various tributaries and empties into the brackish Forcados River that in turn empties into the Atlantic Ocean (fig 1).

To the author's knowledge this represents the first evaluation of diazinon concentrations in water, sediment and fish samples from the Warri River.

2. Study Area and Sampling Schedule

The study area is the Warri River with stations established at Ekakpamre, Ovwian and Ovu (Fig. 1). Ekakpamre and Ovwian stations were chosen to reflect possible sources of pesticides contamination while Ovu Station served as control. Two dominant fish species were selected for this study; *C. furcatus* and *T. zilli*. Catchments of intensive agricultural use are drained into the stations with the exception of the control. The sampling stations were visited on monthly basis. During this period, water, sediment, and fish samples were collected and analyzed for the pesticide residues

2.1. Ovwian Station

This station is surrounded by vegetable and cassava farms, the river receives effluents from Niger Benue Transport Company (NBTC) and Delta Steel Company (DSC), which are located very close to the river

2.2. Ekakpamre Station

The station has poultry farms where diazinon is regularly used for fumigation. The River also drains catchments of intensive agricultural land.

2.3. Ovu Station

This is the upstream station and is located away from agricultural farms. There is no evidence of pesticide usage around this station, which therefore served as the control station.

3. Materials and Methods

3.1. Sample Collection

All samples were collected from designated stations (Ovwian, Ekakpamre and Ovu) of Warri River between Januarys – August 2006. A total of 96 samples made up of 24 samples each for water, sediment and fish (*C. furcatus* and *T. zilli*) were collected and analyzed for diazinon.

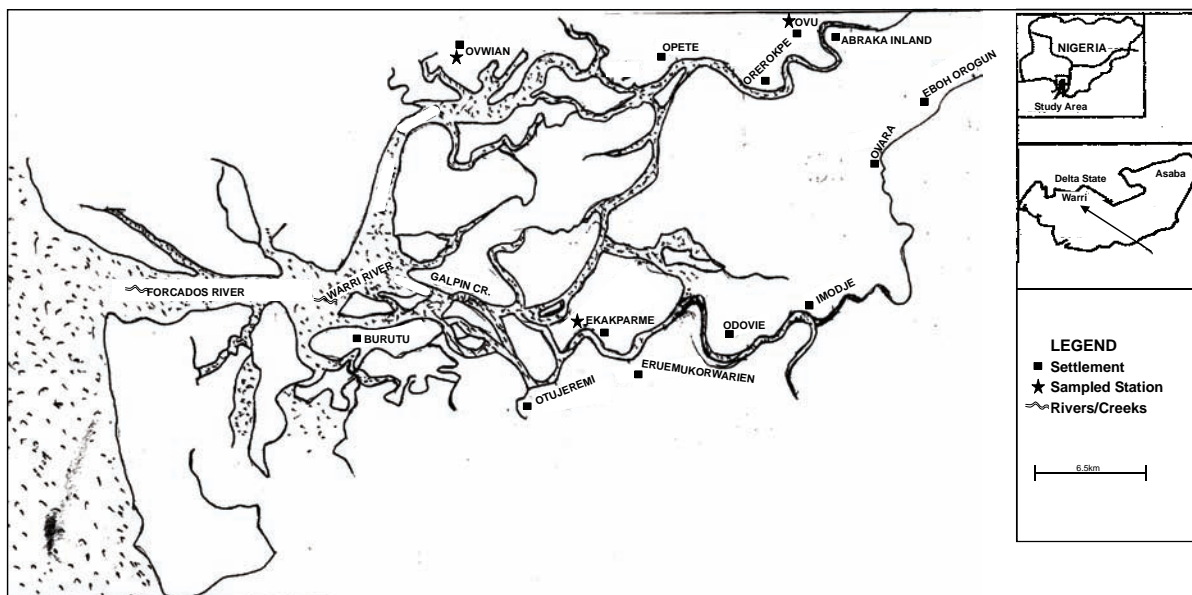


Fig. 1: Map of entire Warri River; Source: Ministry of land and survey, Asaba Delta state (Modified after Ezemonye, 2005)

3.1.1. Water Samples

One liter of water samples were taken from 0.3m below the water surface with a pre – cleaned glass bottle using hydrobios sampler. For sampling, turbulent midstream positions of water bodies were chosen to approximate mean concentration of river water. All foreign bodies were removed and the samples thoroughly homogenized. After collection, the samples were stored on ice during transport (<6hrs) and were kept at 4°C in the laboratory (<2 days) until extraction.

3.1.2. Sediment Samples

Sediment samples were taken from the positions where an accumulation of fine – texture substrate took place. The upper 2cm of bed sediment at each site were collected with a teflon-coated spoon and wrapped in aluminum foil. Samples were immediately stored on ice (<6hrs) after collection and stored at – 20°C in the laboratory until analysis

3.1.3. Fish Samples

Fish samples were captured (1.5-2.5kg/sample, 6samples from each station) wrapped in aluminium foil and kept at 20°C in the laboratory until analysis

3.2. Sample Extraction

The procedure applied for the extraction of pesticides was similar to those reported by; Laab et al., (2000), Steinwandter (1990), and Von Duszejn (1988).

3.2.1. Water Sample

One-liter water sample were extracted using 20ml of hexane: dichloromethane (3:1) for 30 minutes. The extract was concentrated with the aid of rotatory evaporator. Pre-elution was carried out with the HPLC methanol. The concentrated solvent extract was then analyzed for diazinon.

3.2.2. Sediment Samples

Wet sediment samples were homogenized air dried and passed through a no 32 mesh sieve. 15 gram of the sediment were spiked with a solution of surrogate standard (d8-naphthalene, d10-acenaphthene, d12-chrysenes and d12-perylene) and extracted with a mixture of dichloromethane and n – hexane in a ratio 2:3, having been subjected to a vigorous shaking in a sonication bath for 5hrs. The solvent was separated and concentrated with a rotatory evaporator. Pre – elution was carried out with HPLC methanol. The concentrated extract was then analyzed for diazinon.

3.2.3. Fish Samples

Sections of the dorsolateral muscle of fish samples were prepared according to Steinwandter (1990). To estimate the lipid content of the fish, four fish samples were dissected and the lipid tissues isolated. The fish tissues were freeze dried at 60°C Celsius for 4 hours and one gram of dried tissue was weighed into a clean extraction bottle. Diazinon was extracted using acetone, water and dichloromethane. After subjected to vigorous shaking in a solication bath for 5hrs, the solvent was separated, concentrated in a rotatory evaporator and eluted using HPLC methanol. The concentrated extract was then analyzed for diazinon.

3.3. Sample Preparation for Analysis

3.3.1. Chemicals/Reagents

Methanol (HPLC analytical grade), Diazinon (98.5% purity), which were used as internal standard in HPLC analysis were obtained from chemical Service, West Chester, U.K.

3.3.2. Equipment/Glass wares

Cecil High performance Liquid Chromatography (HPLC) system comprised of CE 1200 High performance

variable wavelength monitor and CEII00 liquid chromatography pump, UV detector with variable wavelength and stainless steel column (C₁₈ Reverse phase) packed with Octasilica; vacuum pump and ultrasonic check.

3.3.3. Preparation of External Standard Stock Solution

One milligram per gram stock solutions of the external standard was prepared by measuring 0.1g of diazinon standard into 100ml volumetric flask. A small quantity of methanol was then added to the volumetric flask to dissolve the standard. Distilled water was then used to fill the flask to 100ml mark. The following concentrations (80, 60, 40, 20 and 10) mg/g were later prepared from the 1mg/g stock solution. Then from the 1mg/g concentrations, lower concentrations up to 0.01mg/g were prepared.

3.3.4. Activation of the HPLC System

The target wavelength for the analyses was determined by UV/visible equipment. A small quantity of the stock solution was diluted with methanol and its wavelength of 202nm was determined by scanning. The instrument wavelength was then set at 202nm, with a sensitivity of 0.05nm and a flow rate of 1ml/min. The instrument was purged to remove air and charge the column. Purging was conducted using a washing solution of 30% methanol and 70% distilled water.

3.3.5. Degassing the Mobile Phase Solution

Helium gas was bubbled into the solution to degassing the mobile phase. The mobile phase was then injected into the instrument and allowed to run through the system for 20 minutes. The system was then separated following the procedures outlined in the instrument operating manual.

3.3.6. Determination of Retention Time for External Standard

The internal diazinon standard was injected into the instrument to determine the retention time. A series of concentrations ranging from 0.025ppm to 100ppm were then injected. The resulting peak areas of the resulting chromatographs were plotted against concentrations to determine the linearity of the standard chromatographs. Using this approach, the retention time for the diazinon standard was 4.23 minutes

3.3.7. Pesticide Analysis

Each sample residue was dissolved in 1ml methanol. The extracted residue was then loaded and injected into the valve of the chromatograph system. The resulting chromatograph for each sample was printed out. The retention times noted, concentrations determined and recorded.

3.3.8. Data Analysis

The data were summarized separately for each sampled station using Description statistics (means, range, and histogram). The student's t-test and analysis of variance (ANOVA) was used to test for the level of significance at 0.05 level of probability for the seasons and the stations respectively.

4. Results

Results of spatial and seasonal variations in the concentrations of diazinon in surface water, sediment and fish (*C. furcatus* and *T. zilli*) from Warri River are presented in Tables 1 with further illustration in Figure 2-5.

4.1. Diazinon in Surface Water

The mean diazinon concentrations in the surface water were; 0.93µg/L ±1.05 (Ovwian), 0.41µg/L ± 1.09 (Ekakpamre) and 0.07µg/L±0.19 (Ovu), while the mean value for dry and wet seasons were; 1.76µg/L ± 0.85 and 0.09µ/L ±0.16 (Ovwian), 0.83µg/L±1.49and 0.01µg/L ±0.1 (Ekakpamre), 0.07µg/L ±0.02 and ND(Ovu). The results showed that the pesticide concentrations were significantly different only in the season at P < 0.05 (F=7.61).

4.2. Diazinon in the Sediment

The mean diazinon concentrations in the sediment were; 1.88µg / gdw ±1.26 (Ovwian), 0.78µg / gdw ± 1.45 (Ekakpamre) and 0.28µg/ gdw ± 0.42 (Ovu), while the mean seasonal values for dry and wet seasons were; 2.54µg / gdw ± 0.32 and 1.18µg / gdw ± 1.70 (Ovwian), 1.16µg / gdw ±1.44 and 0.04µg / gdw ±0.03 (Ekakpamre), 0.49µg / gdw ± 0.56 and ND (Ovu) respectively. The concentrations varied significantly both in time and space at P < 0.05 (F = 3.13).

4.3. Diazinon in *C. Furcatus*

The mean diazinon concentrations in this fish were; 2.23µg/ gdw ±2.80 (Ovwian), 1.00µg / gdw ± 0.80 (Ekakpamre) and 0.86µg / gdw ± 1.08 (Ovu), while the mean values for dry and wet seasons were 0.15µg / gdw ±0.03 and 4.29µg / gdw ± 2.62 (Ovwian), 0.11µg / gdw ± 0.08 and 1.66µg / gdw ± 0.39 (Ekakpamre), 0.09µg/ gdw ±0.14 and ND (Ovu) respectively. The pesticide concentrations varied significantly both in time and space at P < 0.05 (F = 3.13).

4.4. Diazinon in *T.Zilli*

The mean values of the pesticide were; 0.29µg / gdw ± 0.37 (Ovwian), 0.04µg / gdw ± 0.05 (Ekakpamre). However, diazinon was not detected in samples collected at station 3. The mean values for dry and wet seasons were; 0.04µg / gdw ± 0.03 and 0.54µg / gdw ± 0.34 (Ovwian), 0.02 µg / gdw ±0.01 and 0.07µg / gdw ±0.05 (Ekakpamre). The concentrations of the pesticide varied significantly both in time and space at P < 0.05 (F = 0.17).

Table 1. Diazinon concentrations during the dry and wet seasons in (a) River water (b) Fine-particle sediments and (c) Fish species in three named sites on the Warri River, Niger Delta, Nigeria sampled monthly from January to August, 2006. Means are based on the monthly observations. ND = Not Detectable.

		Surface Water ($\mu\text{g/gdw}$)		Sediment ($\mu\text{g/gdw}$)		<i>C. furcatus</i> ($\mu\text{g/gdw}$)		<i>Tilapia zilli</i> ($\mu\text{g/gdw}$)	
		Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Dry Season	Ovwian	1.76 \pm 0.85	0.50-2.27	2.54 \pm 0.32	2.16-2.89	0.15 \pm 0.03	0.12-0.19	0.04 \pm 0.03	0.01-0.07
	Ekakpamre	0.83 \pm 1.49	0.02-0.12	1.16 \pm 1.44	0.32-3.31	0.11 \pm 0.08	0.02-0.32	0.02 \pm 0.01	0.01-0.04
	Ovu	0.07 \pm 0.02	0.05-0.09	0.49 \pm 0.56	0.02-1.21	0.09 \pm 0.14	0.01-0.17	ND	
Wet Season	Ovwian	0.09 \pm 0.16	0.01-0.12	1.18 \pm 1.70	0.01-3.64	4.30 \pm 2.62	2.10-7.5	0.54 \pm 0.34	0.13-0.91
	Ekakpamre	ND		0.04 \pm 0.03	0.01-0.07	1.66 \pm 0.39	1.32-2.01	0.07 \pm 0.05	0.01-0.12
	Ovu	ND		ND		ND		ND	

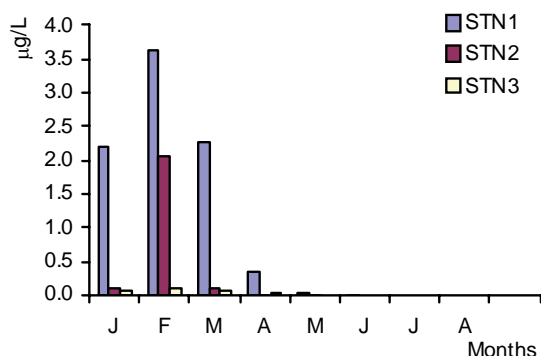


Fig. 2: Monthly variation in surface water diazinon concentrations ($\mu\text{g/L}$) in Warri River

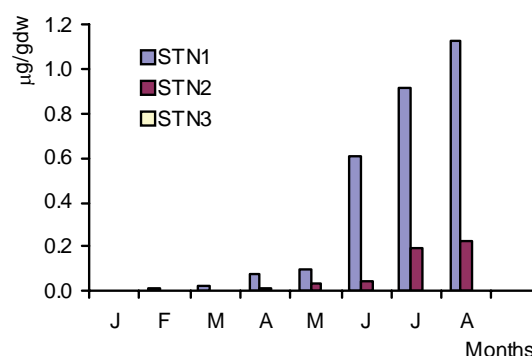


Fig. 5: Monthly variation in *T. zilli* diazinon concentrations ($\mu\text{g/gdw}$) in Warri River

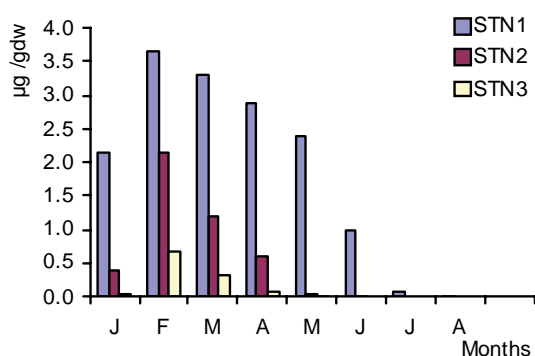


Fig. 3: Monthly variation in sediment diazinon concentrations ($\mu\text{g/gdw}$) in Warri River

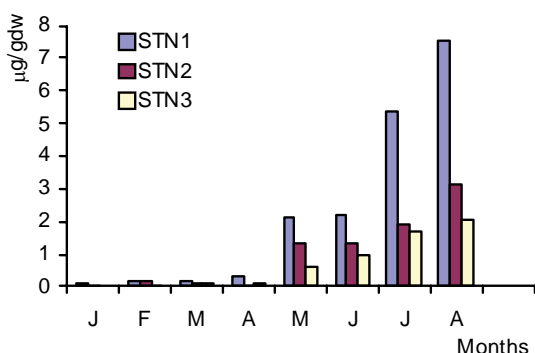


Fig. 4: Monthly variation in *Chrysichthys furcatus* diazinon concentrations ($\mu\text{g/gdw}$) in Warri River

5. Discussion

This study reported the occurrence of varying concentrations of diazinon in surface water, sediment and fish (*C. furcatus* and *T. zilli*) samples from designated stations of the Warri River. Diazinon was detected in all the stations and matrices. It was observed in the station far from agricultural activities with no evidence of pesticide usage except in *T. zilli* tissue where the concentration was insignificant. This is indicative of the ubiquitous use of diazinon outside agricultural usage (Leonard et al., 1999)

Concentrations of diazinon in sediment were much higher than concentrations in water. In general, these results support that concept of sediments acting as a sink for pollutants. The observation corroborated the finding of Voss and Embrey, (2000) where they reported higher concentrations of dichlorvos and malathion in bottom sediment of a small streams toxicity/pesticide study in selected small streams in King and Snohomish Counties, Washington

Higher concentrations of diazinon detected in the fish (*C. furcatus*) from Ovwian and Ekakpamre Stations indicated the long-term effect of agriculture and other human activities. Schmitt et al., (1990), reported similar result during the biomonitoring program of pesticides residues in United State fresh water fish, sediment and water and observed that the concentrations of pesticides compounds detected in fish tissue from eastern Iowa stream and rivers were as a result of long term effect of previous human activities.

Concentrations of diazinon were higher in *C. furcatus* than sediment indicating possible bioaccumulation and

poor elimination in the fish (Wilfred, 1995). Diazinon levels varied significantly between the two fish species (*C. furcatus* and *T. zilli*) at $P < 0.05$ ($F = 4.90$). This may be attributed to lipid content, size and species (Hosteller and Kidwell, 1990). The bottom detritus and predatory feeder *C. furcatus* had diazinon residues significantly higher than the herbivorous *T. zilli*. This variation is probably due to different feeding and living habits. Predatory fishes might bioaccumulate the pesticide by eating other fishes and the constant contact of the fish with the sediment allows their continuous exposure to pesticides. Kent and Johnson, (1974) found that Utah Sucker (*Castomus ardens*) a bottom feeder, contained the highest level of pesticide compounds in American fall Reservoir.

Higher values of diazinon were recorded in the dry season in surface water and sediment samples which is consistent with the observation of Osibanjo and Jensen, (1980) and may be attributed to the planting season (dry season) when farmers treat their farms before planting. Most of the crops grown in these areas (Ovwian and Ekakpamre) are seasonal and include leafy vegetable such as *Amaranthus* species, fluted pumpkin and bitter leaf. Other crops include tomatoes, Okra, sweet pepper, garden eggs and tuber crops like cassava. The concentrations of the diazinon observed in the water and sediment during the rainy season could be as a result of the inactivity during the period in the area.

Lower concentrations of diazinon were observed during the dry season. The pesticide concentrations in (*C. furcatus*) were 53% higher in the raining season than concentrations in the dry season. This could be attributed to; different living habits of the fishes between the seasons, breaking down of the diazinon in environment and absorption of the pesticide through the skin. According to Leight et al., (1999) the accumulation of organic contaminants in the tissue of aquatic organisms is a complex function of the physicochemical properties of the contaminants, its distributions in the aquatic system, the feeding behaviour and metabolism of the aquatic organism.

The concentrations of diazinon increased towards the downstream direction with Ovwian station having highest concentrations. The upstream station (Ovu) was comparatively low with the pesticide contamination; this condition may be due to very low to absence of agricultural activities in the area

6. Conclusion

This study presents the first site-specific data on diazinon concentrations in the Warri River of Niger Delta, Nigeria. It also provides a platform for developing regulatory measures to control contamination of aquatic environments in this region. This is against the background that diazinon levels in Warri river samples from Ovwian, Ekakpamre and Ovu stations exceeded The Environmental Protection Agency (EPA) recommended limit of $0.02 \mu\text{g/L}$ (Hamilton, 2003)

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Effect of Chlorogenic and Caffeic Acids on Activities and Isoenzymes of G6PDH and 6PGDH of *Artemisia Herba Alba* Seeds Germinated for One and Three Days in Light and Dark

(Short Communication)

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Abstract

G6PDH and 6PGDH activities of *Artemisia herba alba* seeds during early stages of seed germination under conditions of light and dark were measured, in the presence of Chlorogenic or Caffeic acids; G6PDH (glucose 6 phosphate dehydrogenase) and 6PGDH (6 phospho gluconate dehydrogenase) activities were inactivated in the presence of both phenolic acids and were stimulated upon germination in the dark (Table 1 and Table 2). This novel approach provides evidence for the variable needs of pentose phosphate pathway activity, depending on concentrations of phenolic acids present and/or light conditions; moreover, a clear response of such germination condition was earlier expression of a second isoenzyme for G6PDH in the presence of 0.4 mM Chlorogenic or Caffeic acids with apparently variable inhibitory pattern.

المخلص

لقد اجري هذا البحث لدراسة تأثير اثنين من الأحماض الفينولية (الكافيك و الكلوروجيك) على نشاط إنزيمي جلوكوز ٦ فوسفات ديهيدروجينيز و ٦-فوسفات جلوكونيت ديهيدروجينيز المستخلصة من بذور نبات الشيح النامية في ظروف الضوء و الظلام لمدة ١ أو ٣ أيام في مستويات مختلفة من الحوامض الفينولية، وقد تبين أن نشاط الإنزيمين يزداد من اليوم الأول إلى الثالث في ظروف الضوء و الظلام، كما أن الإنزيمين يتم تثبيطهما في وجود حامضي كلوروجينيك و كافيك في ظروف الضوء و الظلام. وقد بينت النتائج الحاجة المتغيرة لنشاط تفاعلات سلسلة السكريات الخماسية المفسفرة في وجود حامضي كلوروجينيك و كافيك. أما بالنسبة إلى أشباه الإنزيمين فقد بينت النتائج وجود شبيه واحد في اليوم الأول ثم يصبح شبيهين اثنين في اليوم الثالث من نمو البذور لكلا الإنزيمين في الظلام. كما بينت الدراسة أن وجود الأحماض الفينولية عند الإنبات يؤدي إلى الظهور المبكر لشبيه جلوكوز ٦ فوسفات ديهيدروجينيز كما يؤثر على احد الشبيهين أكثر من الآخر.

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Keywords: Chlorogenic acid, Caffeic acid, G6PDH, 6PGDH, *Artemisia herba alba*;

1. Introduction

Phenolic compounds were linked with seeds germination inhibition and dormancy in *Artemisia herba alba*. (Al-Charchafchi and Al-Quadan, 2006, Al-Charchafchi et al., 1987; Alam et al., 2001; Muscolo et al., 2001). Their concentrations in plants were subject to seasonal alterations (Solar et al., 2006). Phenolics accumulation played a protective role in strengthening the plant cell walls during growth by polymerization into lignins (Diaz, 1997).

Phenolic compounds are primarily synthesized through the pentose phosphate, and shikimate pathway. (Ali et al., 2006, Sgherri et al., 2004, Dixon, and Paiva 1995, Shetty et al., 2002; Randhir et al., 2005, Kovačik et al., 2006).

Glucose-6-phosphate dehydrogenase (G6PDH E.C 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH E.C 1.1.1.44) are the key enzymes of pentose phosphate pathway, used in the biosynthesis of phenolic compounds (Andrei et al., 2002; Sükrü et al., 2003). In germinated seeds of *Artemisia herba alba*, activities of both enzymes were inhibited in presence of esculetin (Al-Charchafchi and Al-Quadan, 2006); whereas elevation of both activities were noticed in the dark (Robinson, 2000).

This study was undertaken to investigate G6PDH or 6PGDH activities in seeds of *Artemisia herba alba* germinated under the dual effect of inhibition of both enzyme activities (growth in presence of phenolic compounds), and stimulation of both enzyme activities, that is the growth under dark condition; The timing of this study was designed at one and three days of germination, which represents an early stage of photosynthetic system development, and hence halt of NADPH₂ production from

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photosynthetic system; moreover, isoenzyme patterns of both dehydrogenases under such conditions were also investigated.

2. Materials and Methods

2.1. Plant Material

Seeds of *Artemisia herba alba* used in the study were harvested during December 2003 from the medicinal and aromatic plant garden of the Hashemite University/Zarka, Jordan. The seeds were kept in brown paper bags in refrigerator until used.

2.2. Germination and Growth

Germination was performed in 12 cm diameter petri dishes lined with one layer of filter paper (Whateman No.30) and moistened with 5 ml of distilled water or Chlorogenic or Caffeic acids at 0.2 and 0.4 mM. 100 seeds per dish (three replicates) were used for each treatment. All dishes were incubated at 20 °C in growth chamber under continuous illumination or darkness for 1, 3, and 5 days. Dark treatment was conducted by wrapping the dishes with aluminum foil immediately after seed imbibitions. Chlorogenic and Caffeic acids were prepared just prior to use and were wrapped in aluminum foil container.

2.3. G6PDH and 6PGDH Extraction Procedure

Extraction solution consisted of 100 mM Tris-HCl (pH 7.6), 2 mM EDTA Na₂ [Scharlau, Spain] and 30 mM β -Mercaptoethanol [ARCOS, USA]. The imbibed seeds and seedlings were suspended in 1 ml of extraction solution and were homogenized at 25000 rpm for 3 min using IKA homogenizer [Germany]. During homogenization, samples were immersed in ice bath. Homogenates were then centrifuged for 20 minutes at 10000 rpm at 4°C; supernatant were re-centrifuged two more times for 5 minute to remove lipid debris. Supernatants were used immediately for G6PDH and 6PGDH activity determination, or stored at -70 °C until time for analysis.

2.4. G6PDH and 6PGDH Assays

The assay procedure as described earlier (Al-Quadan and Al-Charchafchi 2006) was used. All assays were performed with GENSYS 5, USA spectrophotometer. Each assay consisted of 850 μ l Tris-HCl buffer (10 mM, pH 7.6), 100 μ l MgCl₂ (67 mM), 10 μ l glucose-6-phosphate or 6-Phosphogluconate (10 mM) [Sigma, Mo, USA] and 10 μ l NADP⁺ (5mM) [BDH, England] in a total volume of 1 mL.

2.5. Protein Determination

Quantitative protein determination by the method of Bradford (1976), with bovine serum albumin (BSA) was used as standard.

2.6. Electrophoresis

Polyacrylamide gel electrophoresis and isoenzymes visualization was carried out as described earlier (Al-Quadan and Al-Charchafchi 2006) using 7.5% acryl amide and Bio-Red vertical cell [USA]. The gels were photographed using digital camera (JAI, Japan) interfaced to a computer [DELL, USA].

3. Statistical Analysis

ANOVA test was used to determine the level of significance within the *Artemisia herba alba* species regarding the effect of Chlorogenic acid or Caffeic acid on G6PDH and 6PGDH activities. Significance of differences was accepted when $p < 0.05$.

4. Results and Discussion

G6PDH and 6PGDH activities after one, and three days of seed germination under light or dark condition, in the presence or absence of different concentrations of Chlorogenic and Caffeic acids are presented in Tables 1 and 2.

G6PDH and 6PGDH activities were increased from day 1 to day 3 of growth under growth condition used ($P < 0.05$). On the other hand, in the presence of 0.2 mM or 0.4 mM Chlorogenic or Caffeic acids both activities were significantly decreased ($P < 0.05$) in comparison with absence of such phenolic compounds; the inhibition pattern was less pronounced on day three of seed germination; our explanation to this inhibition pattern may account either for the differential inhibition or sub cellular distribution of both isoenzymes. (Debnam and Emes (1999); Al-Quadan *et al.*, 1982; Hoover *et al.*, 1977). Chlorogenic and Caffeic acids inhibitory effect on the germination of *Artemisia herba alba* seeds (Al-Charchafchi and Al-Quadan, 2006) was strongly correlated to the inhibition of the very sensitive enzyme activities of oxidative pentose phosphate pathway.

The elevated activities of pentose phosphate pathway on day three in seeds germinated in the dark may be needed to compensate the short supply of NADPH resulted from the halt of photosynthetic pathway (Robinson, 2000). Number of isoenzymes of G6PDH and 6PGDH after one, and three days of seed germination under light or dark condition, in the presence or absence of different concentrations of Chlorogenic and Caffeic acids are presented in Table 3.

After one day of seed germination under light or dark condition, electrophoresis study showed only one G6PDH isoenzyme band (R_f 0.52); an additional slower isoenzyme band (R_f of 0.49) was present after three days of seeds growth in the presence of 0.4mM Chlorogenic or Caffeic acids. This slower band was also present on the third day of growth under light condition, but only if 0.4 mM Chlorogenic or Caffeic acids are present. The slow moving band had a lower relative intensity compared to the fast moving band.

Electrophoresis study showed one 6PGDH isoenzyme band (R_f 0.50) after day one of germination; an additional

faster isoenzyme band (R_f of 0.53) was present after three days of seed germination under all growth condition used.

The fast moving band had lower relative intensity compared to the slow moving band

Table1: G6PDH specific activities (Units/mg protein) of *Artemisia herba alba* seeds after one and three days of germination under growth conditions indicated in presence of Chlorogenic acid or Caffeic acid. Values reported were mean of triplicate repeats \pm SD

mM Chlorogenic or Caffeic acid	Chlorogenic acid				Caffeic acid			
	Light Treatment		Dark Treatment		Light Treatment		Dark Treatment	
	Day 1 G6PDH	Day 3 G6PDH	Day 1 G6PDH	Day 3 G6PDH	Day 1 G6PDH	Day 3 G6PDH	Day 1 G6PDH	Day 3 G6PDH
0	44 \pm 11	49 \pm 9	47 \pm 12	70 \pm 13	44 \pm 11	49 \pm 9	47 \pm 12	70 \pm 13
0.2	32 \pm 8	36 \pm 11	41 \pm 12	60 \pm 9	26 \pm 6	32 \pm 7	42 \pm 13	64 \pm 12
0.4	34 \pm 8	39 \pm 13	41 \pm 12	63 \pm 12	36 \pm 9	42 \pm 10	45 \pm 12	65 \pm 11

Table 2: 6PGDH specific activities (Units/mg protein) of *Artemisia herba alba* seeds after one and three days of germination under growth conditions indicated in presence of Chlorogenic acid or caffeic acid

mM Chlorogenic or Caffeic acid	Chlorogenic acid				Caffeic acid			
	Light Treatment		Dark Treatment		Light treatment		Dark Treatment	
	Day 1 6PGDH	Day 3 6PGDH	Day 1 6PGDH	Day 3 6PGDH	Day 1 6PGDH	Day 3 6PGDH	Day 1 6PGDH	Day 3 6PGDH
0	47 \pm 13	61 \pm 10	62 \pm 10	69 \pm 11	47 \pm 13	61 \pm 10	62 \pm 10	69 \pm 11
0.2	24 \pm 6	62 \pm 11	38 \pm 8	62 \pm 13	35 \pm 7	48 \pm 11	45 \pm 6	53 \pm 11
0.4	38 \pm 11	67 \pm 14	51 \pm 10	67 \pm 10	41 \pm 9	58 \pm 11	52 \pm 9	61 \pm 11

Table 3: Effect of Chlorogenic /Caffeic acid on number of G6PDH and 6PGDH isoenzymes from *Artemisia herba alba* seeds after 1 and 3 days of germination under light and dark condition.

Chlorogenic or Caffeic acid mM	G6PDH isoenzymes				6PGDH isoenzymes			
	Light Treatment		Dark Treatment		Light treatment		Dark Treatment	
	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3	Day 1	Day 3
0	1	1	1	2	1	2	1	2
0.2	1	1	1	2	1	2	1	2
0.4	1	2	1	2	1	2	1	2

This study has indicated that activation and inhibition of pentose phosphate pathway enzyme activities in *Artemisia herba alba*, provides evidence for the variable needs of pathway activity depending on light conditions and /or concentrations of phenolic compounds presence; moreover, the early expression of second G6PDH isoenzyme as early as day three of germination in the presence of Chlorogenic or Caffeic acids, and with apparently different inhibitory patterns, elucidated the role played by those phenolic compounds in regulating pentose phosphate pathway activity.

Future study of Chlorogenic and Caffeic acids effect on purified isoenzymes should provide insight into this differential inhibition pattern.

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