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Shao R and Barker SC. 2007. Mitochondrial genomes of parasitic arthropods: implications for studies of population genetics and evolution. Parasit. **134**:153-167.

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Embabi NS. 1990. Environmental aspects of distribution of mangrove in the United Arab Emirates. Proceedings of the First ASWAS Conference. University of the United Arab Emirates. Al-Ain, United Arab Emirates.

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El-Labadi SN. 2002. Intestinal digenetic trematodes of some marine fishes from the Gulf of Aqaba (MSc thesis). Zarqa (Jordan): Hashemite University.

f. In press articles:

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Influence of Culture Conditions on Cellulase Production by *Streptomyces* Sp. (Strain J2)

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Abstract

The purpose of this study was to determine the influence of growth conditions and medium composition on the cellulase enzyme production by *Streptomyces sp.* Production of cellulase enzyme by a *Streptomyces* strain (J2) was detected on cellulose agar (CA) medium after 4 days of incubation at 28 °C that exhibited a clear zone of 22 mm around the colony. Cellulase production was assayed by measuring the amount of glucose liberated in µmol/ml/min by using the dinitrosalicylic acid assay method. The highest crude enzyme activity (432 U/l) was observed after 3 days of incubation at pH 7 and 60 °C in a medium that was supplemented with 0.5% glucose, 0.2% starch, and 0.2% NH_4CL. However, enzyme production and activity were strongly decreased at 45°C and acidic pH. Enzyme production and activity were also inhibited when *Streptomyces* strain (J2) was grown in CMC broth supplemented with arabinose and yeast extract as a sole carbon and nitrogen source, respectively.

الملخص

كان الهدف من هذا البحث دراسة تأثير ظروف النمو وتركيب الوسط الغذائي على إنتاج إنزيم السليليز من بكتيريا الـ *Streptomyces*. تم الكشف على إنتاج انزيم السليليز من بكتيريا الـ *Streptomyces* بعد حضنها لمدة أربعة أيام على 28 درجة مئوية وفي وسط غذائي يحتوي على السليلوز وتم تقدير إنتاج الإنزيم بقياس الدائرة الشفافة حول مستعمرة البكتيريا والذي يعني أن الإنزيم افرز وانه قد حطم السليلوز المحيط بالمستعمرة. كذلك تم قياس نشاط الإنزيم المنتج بواسطة قياس كمية الجلوكوز المنتج عند استعمال طريقة DNSحيث سجل اكبر نشاط للأنزيم (432 وحدة) بعد ثلاثة أيام من الحضن على درجة حموضة 7 ودرجة حرارة 60 مئوية في وسط يحتوي على 0.5 % جلوكوز، و 0.2% نشأ، و 0.2 % NH_4CL . بدأ إنتاج ونشاط الإنزيم بالهبوط على درجة حرارة 45 مئوية ودرجة حموضة حامضية. لقد لوحظ كذلك أن نشاط الإنزيم قد انخفض بشدة عندما زرعت العزلة J2 في وسط يحتوي على سكر الارابينوز كمصدر وحيد للكربون ومستخلص الخميرة كمصدر وحيد للنيتروجين.

Keywords: Conditions; Culture; Cellulase; *Streptomyces* Sp

1. Introduction

A wide variety of bacteria are known for their production of hydrolytic enzymes with streptomycetes being the best known enzyme producers (Vinogradova and Kushnir, 2003). They are capable of secreting an array of different extracellular enzymes including cellulases, chitinases, and xylanases. Actinomycete's, one of the known cellulase-producers, has attracted considerable research interest due to its potential applications in recovery of fermentable sugars from cellulose that can be of benefit for human consumption and to the ease of their growth (Jang and cheng, 2003) compared to anerobic cellulase producers such as *Paenibacillus curdlanolyticus* (Pason *et.al*. 2006). The biotechnology applications of cellulases began in the early 1980s in animal feed followed by food applications (Harchand and Singh, 1997). Today, these enzymes account for approximately 20% of the world's enzyme market.

Few studies have been conducted on Streptomyces isolated from Jordan soil for their potential to produce enzymes of industrial importance. Rawashdeh et. al. (2005) isolated several Streptomyces isolates that were able to grow on tomato pomace. Upon further characterization, these isolates were able to produce cellulase, pectinase, and relatively large amount of xylanase. Tahtamouni et. al.(2006) isolated indigenous Streptomyces isolates that were capable of producing chitinase. These isolates exhibited fungicidal activity against sclerotia of the white cottony stem rot pathogen Sclerotinia sclerotiorum. Streptomycetes isolated from Jordanian habitats are poorly studied, especially the cellulase enzyme producers. Therefore, the present investigation is conducted to isolate soil streptomycetes from different habitats in Jordan, and to screen them for their ability to utilize cellulose as a sole source of carbon in an attempt to isolate a highly active isolate in cellulose production that cab used in the partial degradation of plant fibers, and thus cab be of industrial quality. The influence

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of different culture conditions on production of crude cellulase by the most active Sterptomyces isolate in submerged cultures is also studied.

2. Materials and Methods

2.1. Location, Sampling, Treatment of Soil Samples, and Isolation Technique

Twenty Soil samples were collected from 20 different regions in Jordan (Table 1). Enrichment of streptomycetes in the soil samples and isolation of *Streptomyces* spp. were performed as described by Saadoun *et. al.*(2008). Selected colonies were purified by repeated streaking. *Streptomyces*-like colonies were selected and screened for their ability to produce cellulase enzyme on cellulose agar medium (Carder, 1986).

2.2. Screening for Cellulase-Producing Streptomyces

Each isolate was suspended in sterile vial containing 3 ml distilled water, to create a spore suspension of 107 spores/ml. A drop (0.1 ml) from the suspension was cultured on the center of a cellulose agar (CA) plate (Carder, 1986). Pure Streptomyces isolates were cultured onto CA plates and incubated for 4 days at 28° C. Plates were then flooded with 0.1% Congo red for 15-20 min, then washed with 1ml NaCl, and kept over night at 5 °C. Bacterial colonies exhibiting clear zones against red color of non-hydrolyzed media were considered cellulase producers; and were tested again for confirmation (Carder, 1986).

2.3. Characterization of The Most Active Streptomyces Isolates

Streptomyces colonies that showed the largest clear zone were characterized morphologically and physiologically according to the International *Streptomyces* project (ISP) (Shirling and Gottlieb, 1966) and as described by Saadoun *et al.* (2008).

2.4. Cellulase Activity Assay

Cellulase activity was measured following the method of Miller (1959). Briefly, a reaction mixture composed of 0.2 ml of crude enzyme solution plus 1.8 ml of 0.5% carboxymethyl cellulose (CMC) in 50 mM sodium phosphate buffer (pH 7.0) was incubated at 37 °C in a shaking water bath (GFL, Germany) for 30 min. The reaction was terminated by adding 3 ml of DNS reagent. The color was then developed by boiling the mixture for 5 min. Optical densities of samples were measured at 575 nm against a blank containing all the reagents minus the crude enzyme. Results were then compared to controls inoculated with an in active cellulytic streptomycete isolate. Results were interpreted in terms of enzyme activity in which one unit (U) of enzyme activity was defined as the amount of enzyme, which liberates 1μmol of glucose per minute under the above assay conditions (Miller, 1959).

2.5. Optimization of Growth Conditions and Cellulase Production

Erlenmeyer flasks containing 50 ml of CMC broth medium (Per liter: CMC: 10 g; KH_2PO_4: NaCl: 2 g; $MgSO_4.H_2O$: 1 g; $MnSO_4$:0.05 g; $FeSO_4.7H_2O$: 0.05 g $CaCl_2.2H_2O$: 2 g; NH_4Cl:2 g; pH 7-7.4) were inoculated with 1 ml of spore suspension (10^7spores/ml) of a 7 days old culture. Cultures were incubated in an orbital shaker incubator (TEQ, Portugal) at 28^{o}C for 5 days. Cellulase activity was then assayed daily by the DNS method as described above.

Table 1. Geographical locations from which soil samples were collected and designation of samples.

Location	Designation of samples	Latitude*	Longitude*
Safawi	Sa	32°10′N	37^{0}07′E
Mafrak	Ma	32°21′N	36^{0}12′E
Jafr	Ja	30°17′N	36^{0}20′E
Azraq	Az	31°50′N	36^{0}49′E
Ruwayshid	Ru	30°17′N	36^{0}07′E
Dayr abu sa'id	Da	32°^{0}30′N	35^{0}41′E
Mazar	Mz	31°04′N	35^{0}42′E
Marrow	Mr	32°37′N	35^{0}53′E
Tayiba	Ty	32°33′N	35^{0}35′E
Al-esheh	Esh	-	-
El-Ne`aemi	En	32°25′N	35^{0}55′E
En nabi	Na	32°04′N	35^{0}43′E
Hamamet e`lemat	Ha	32°24′N	35^{0}43′E
Kafr khall	KA	32°22′N	35^{0}53′E
Ba'un	Ba	32°23′N	35^{0}44′E
Raymun	Ra	32°17′N	35^{0}50′E
Jordan University of Science andTechnology	Just	32°34′N	36^{0}00′E
Turrah	Tu	32°38′N	35^{0}59′E
Shajarah	Sh	32°39′N	35^{0}56′E
Thnebeh	Th	32°40′N	35^{0}57′E
Total number of samples		20	

*Gazetteer of Jordan.

2.6. Effect of Ph and Temperature on Cellulase Production

To study the effect of pH and temperature on cellulase production, 250 ml Erlenmeyer flasks were prepared containing 50 ml of 0.5% (W/V) CMC broth, with pH values in the range of (4-9). Buffers including sodium acetate (pH 4), citrate buffer (5, and 6), phosphate buffer (pH 7), Tris buffer (pH 8 and 9) at final concentration of 50 mM were used to adjust the pH of the broth. Flasks were then incubated at 28 °C for the optimum incubation time. Samples from bacterial cultures growing in these broths were assayed daily for cellulase production using the standard DNS method (Miller 1959). The pH value giving the highest enzyme production was used for further enzyme assays.

The optimal temperature for enzyme production was determined by performing the standard assay procedure at range temperatures of 15 to 45 °C in an orbital incubator shaker (TEQ, Portugal). All further enzyme assays were performed at the determined optimum conditions (pH and temperature).

2.7. Effect of Various Carbon Sources on Cellulase Production.

To study the effect of various carbon sources on the enzyme production, 250 ml Erlenmeyer flasks were prepared containing 50 ml of mineral salts medium supplemented with 0.2% (W/V) of one of the following carbon sources: glucose, arabinose, starch, glycerol, citrus pectin, and CMC giving a total of 0.5% carbon source. Cultures were incubated at the determined optimum conditions (time, pH and temperature), and the activity of the enzyme was assayed as described above.

2.8. Effect of Various Nitrogen Sources on Cellulase Production

Different organic and inorganic nitrogen sources were tested for their effect on the enzyme production, following the same procedure used for testing the effect of carbon sources on cellulase production. Sources including KNO_3, NH_4Cl, peptone, asparagines, and yeast extract were used in this study. The activity of the enzyme was assayed as above.

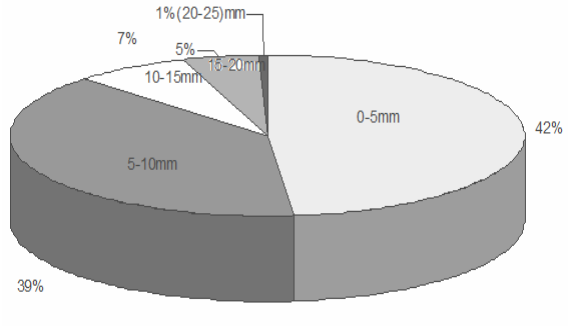


Figure 1. Distribution of the CMC degrading Streptomyces isolates.

2.9. Effects of The Combination of Ph and Temperature on Cellulase Production

This experiment was designed to test the effect of the combination of both temperature and pH on cellulase activity. Buffers of sodium acetate (pH 4 and 4.5), citrate (pH 5, 5.5 and 6), phosphate (pH 6.5 and 7), and Tris (pH 8 and 9) at 50 mM were used in this study to prepare 0.5% (W/V) CMC solution. Culture filtrates were used as crude enzyme source. Enzyme production was assayed as described eearlier at a wide range of temperatures from 4 to 100 °C.

2.10. Cellulase Production Under Optimum Conditions

The most active *Streptomyces* sp. (strain J2) was cultivated in CMC broth medium with the optimized incubation conditions (time, incubation temperature, pH, and carbon and nitrogen sources). Crude cellulase enzyme produced by this active strain under these optimal conditions was assayed as mentioned earlier.

2.11. 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 $\alpha = 0.05$.

3. Results

By employing enrichment methods, a total of 340 different *Streptomyces* isolates were recovered from 20 soil samples, which were collected from different habitats in Jordan. All of these isolates matched the genus description reported by Shirling and Gottlieb (1966), Nonomura (1974), and Williams *et al*., (1983).

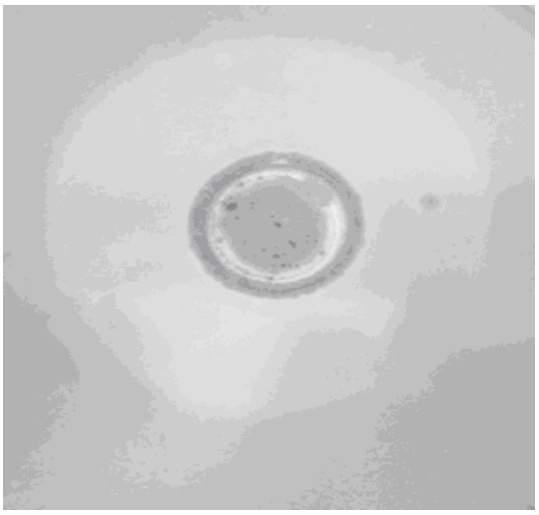


Figure 2. Cellulytic activity of *Streptomyces* sp. (strain J2) on cellulose agar (CA) indicated by clearing zone surrounding the colony.

Upon initial screening, it appeared that most of the isolates (94%) were able to produce cellulase enzyme (Figure 1) while only 6% of the isolates were unable to produce this enzyme. Cellulase producing isolates were categorized into 5 groups according to the width of inhibition zones; very strong (group 1), strong (group 2), moderate (group 3), weak (group 4), and very weak (group 5). Zones of inhibition were 20-25, 15-20, 10-15, 5-10, and 0-5 mm for the 5 groups, respectively. The first 3 groups were represented by 1, 5, and 7% activity, respectively, while groups 4 and 5 were represented by 39 and 42% activity, respectively (Figure 1).

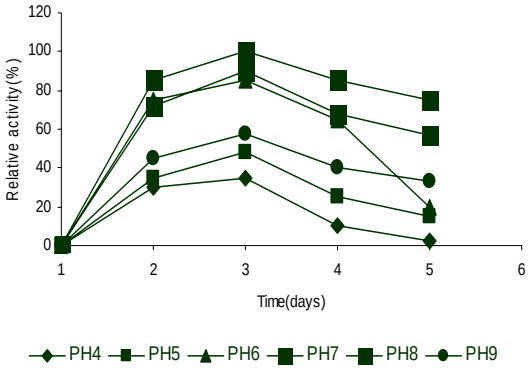


Figure 3. Determination of optimum pH for CMCase production by the J2 isolate. Enzyme activities at the different conditions are compared to the highest value, considered as 100%.

The isolate J2 was chosen as the most active cellulose-degrading isolated *Streptomyces*, which exhibited a 22 mm diameter of clear zone on CA plates (Figure 2).

Morphological and physiological characterization of this strain (J2) revealed that it belonged to the white color series with a distinctive reverse side color (light brown). In addition, this isolate did not produce diffusible and melanin pigments; and had a rectiflexible (RF) sporophore arrangement. The isolate was unable to utilize I-inositol and rhaminose. However, it utilizes other sugars such as D-glucose, L-arabinose, D-xylose, D-fructose, sucrose, D-mannitol, and raffinose.

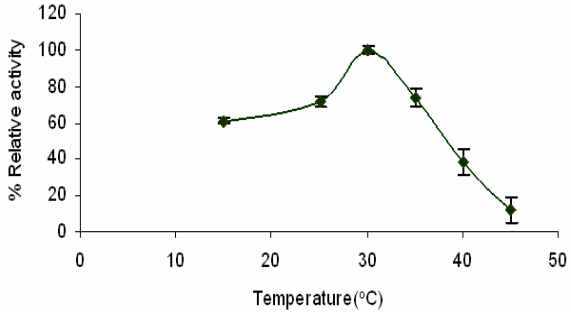


Figure 4. Determination of the optimum temperature for CMCase production by the J2 isolate. Enzyme activities at the different conditions are compared to the highest value, considered as.

Figure (3) shows that J2 strain minimally produce cellulase at pH 4 and 5 with relative activities of 35% and 48%, respectively, whereas more production was observed at pH 7 with relative activity of 100% after 72 h of incubation. Enzyme relative activity eaquals its activity at that specific condition when compared to the optimal (100%). Relative activities at pH 7 were not significantly different (P>0.05) from those activities at pH 8, but they were significantly (P>0.05) from the relative activities at pH 9 at, which the enzyme production barely exceeds 50%. Optimum enzyme production was observed after 3 days at 30°C (Figure 4) with 100% relative activity, which decreased considerably at 45 °C reaching only 12%.

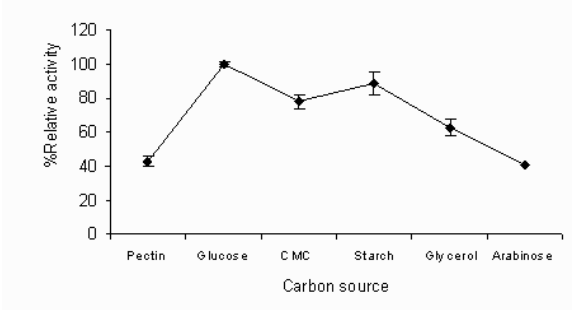


Figure 5. Effect of different carbon sources on CMCase production by J2 isolate. Enzyme activities at the different carbon sources are compared to the highest value, considered as 100%. Pars represent the standard errors for means at α=0.05.

Surprisingly, when observed in Figure (5), the maximal CMCase production by J2 was observed when 2% W/V glucose was used as a carbon source with a 100% relative activity. However, when pectin or arabinose was used, a significant decrease in the relative activity reaching 43%, 40% for pectin and arabinose, respectively was observed.

The highest level of enzyme production was achieved when NH_4CL was added to the CMC medium as sole source of nitrogen while the lowest yield was observed when yeast extract and asparagines were used as nitrogen sources yielding relative activities of 35% and 55%, respectively (Figure 6).

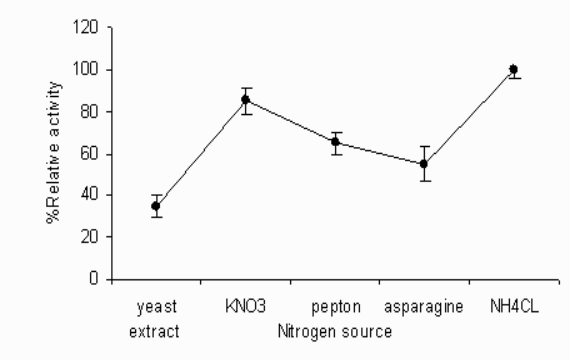


Figure 6. Effect of different nitrogen sources on CMCase production by J2 isolate. Enzyme activities at the different nitrogen sources are compared to the highest value, considered as 100%. Pars represent the standard errors for means at α=0.05.

The temperature and pH profiles are presented in (Figure 7). CMCase have shown more than 40% activity at all pH values tested with maximum production at pH 6 (Figure 7a). An optimum temperature (plateau) ranging from 45 to 60°C was observed (Figure 7b) with maximum production observed at 60°C. It is noteworthy that at 45°C the enzyme production still retained 80% of its activity. The highest level of cellulase activity (432 U/L) was observed under optimum conditions after 3 days of incubation at pH 7 (Figure 8).

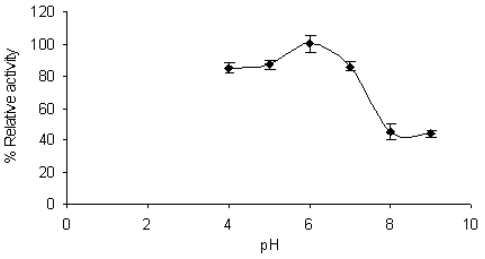
4. Discussion

Streptomyces species have been always a source of thousands of bioactive compounds. Enzymes are one of the important products of this unusual group of bacteria. Most of the Streptomyces isolates recovered from the different soils of Jordan produced fiber hydrolytic enzymes. Cellulase, one important hydrolytic enzyme, was produced by most of the isolates (94%).

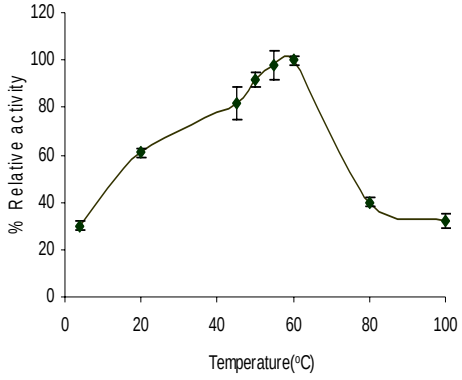
Figure 5. Effect of different carbon sources on CMCase production by J2 isolate. Enzyme activities at the different carbon sources are compared to the highest value, considered as 100%. Pars represent the standard errors for means at α=0.05.

CMCase enzyme from the active isolate J2 was found active over a pH range of 4-7 with maximum activity at pH 6. This result is considerably similar to what was reported by Theberge et. al. (1992) who showed that the optimum pH for endoglucanase from a strain of Streptomyces lividans was 5.5. However, the results appeared to contradict previous results reported by Solingen et al. (2001) of an alkaline novel Streptomyces species isolated from east African soda lakes that have an optimal pH of 8, highlighting the effect of alkaline environment on the adaptation of these Streptomyces. Furthermore, the maximum CMCase activity of isolate J2 was recorded at 60 °C with no significant difference ($p<0.05$) between 50 and 60°C. These results are in agreement with results reported by McCarthy (1987), who reported an optimal temperature for cellulase activity in the range of 40-55 °C for several Streptomyces species including S. lividans, S. flavogrisus, and S. nitrosporus. Jang and Chen (2003) described a CMCase produced by a Streptomyces T3-1 with optimum temperature 50 °C,

whereas Schrempf and Walter (1995) described a CMCase production by a S. reticuli at an optimum temperature 55 °C.



(a)



(b)

Figure 7 . Effect of pH (a) and temperature (b) on CMCase activities, Enzyme activities at the different conditions are compared to the highest value, considered as 100%.

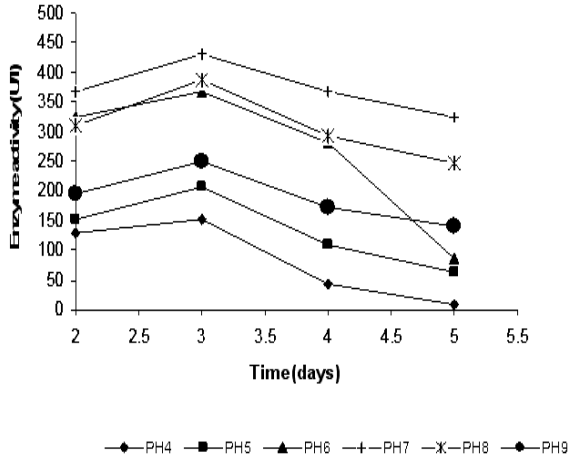


Figure 8. Course of cellulase production by J2 isolate at different pH values. Cellulase assayed when a mixture of glucose (0.5%) plus starch (0.2%) were used as a carbon sources and NH_4CL (0.2%) as a nitrogen source and temperature at 30 °C.

Contrary to the described pattern for other Streptomyces family members, our results indicates that cellulase production by J2 strain was not negatively affected by glucose. A result that may confer an economical advantage for this strain. However, there is no significant difference between glucose and CMC in the induction of cellulase production. It has been reported that the biosynthesis of cellulase is induced during growth on cellulose or other cellulose derivatives (Fernandez-Abalose et.al.. 1997; Godden et. al. 1989). In all cases, it has been found that it is essential to keep the required nutrients at low level to insure maximum accumulation of fermentation products (Priest, 1984). Overall, the study indicated that cellulase production from J2 isolate was constitutive in nature, as apparent from the very high number of Streptomyces isolates producing CMCase in Jordan soils.

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Attempts for Detection of Nanoparticles-Nanobacteria and Distribution of their Antibodies in Jordanian Patients with Urolithiasis

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Abstract

In 1998, calcifying nanoparticles (CNPs) or nanobacteria was proposed as an explanation of certain kinds of pathologic calcification and stone formation. In the present study Enzyme Linked Immunosorbent Assay (ELISA), bacterial culturing, and staining techniques were used to investigate the incidence of calcifying nanoparticles IgG antibodies (anti-CNP Abs) in serum of Jordanian patients with urolithiasis and the living nature of these CNPs in the renal stones. Serum samples from 65 patients and related 20 healthy individuals were tested for anti-CNP Abs. Renal stones retrieved from the kidneys of 20 patients were processed and subjected to mammalian cell culture conditions, then bacterial growth and staining were observed from these cultures. Results revealed detection of anti-CNP Abs in 96% of patients and in 40% of healthy individuals. Although high anti-CNP Abs incidence were correlated strongly with the presence of CNPs and urolithiasis, no CNPs or bacterial growth was detected - following the applied staining and turbidity methods, which may reflect the non living nature of such particles. The findings of this study can be used as a tool for early prediction of kidney stone formation.

الملخص

في عام 1998 تم اكتشاف الجزيئات المتكلسه المتناهيه في الصغر أو الجراثيم المتناهية في الصغر(النانوبكتيريا). وارتبطت هذه الجزيئات المتكلسه ارتباطا مباشرا بتكوين حصى الكلى وحدوث الأمراض المزمنة ذات الطبيعة التكلسية. في هذا البحث تمت دراسة هذه الجزيئات باستخدام الطرق المناعية لتحديد الأجسام المضادة لها في عينات مصل المرضى كما تم زراعه عينات حصى الكلى في الأوساط الزراعية الخلوية لتحديد الطبيعة الحية لتلك الجزيئات المتكلسه. تم جمع 65 عينه دم من المرضى الأردنيين المصابين بحصى الكلى وكذالك 20 عينه دم من أناس أصحاء لاستخدامها كعينة سيطرة. تم فحص المصل لتلك العينات لتحديد الأجسام المضادة ضد تلك الجزيئات. كما تم جمع 20 عينه حصى كلى وتمت زراعتها في أوساط خلوية وتم مراقبه تلك الزروعات ومحاولة صباغتها لتحديد أي نمو بكتيري. تم تحديد الأجسام المضادة لتلك الجزيئات المتكلسه في 96% من عينات المرضى وفي 40% من عينات السيطرة. لم نتمكن من تكثير تلك الجزيئات المتكلسه في الأوساط الخلوية ولم تعطي محاولات الصباغة أي دليل لنمو بكتيري في تلك الزروعات. بالرغم من عدم زراعة تلك الجزيئات المتكلسه في الأوساط الخلويه إلا أن النسبه العاليه للأجسام المضاده المكتشفه لها في عينات المرضى وحتى في عينات الأصحاء تعكس الرابط القوي على وجود تلك الجزيئات والإصابه بالأمراض المختلفه ذات الطبيعه التكلسيه. ان نتائج هذه الدراسه تبين امكانية استخدام تحديد الأجسام المضاده في عينات المرضى لتلك الجزيئات كوسيله للتشخيص المبكر للأمراض المختلفه ذات الطبيعه التكلسيه مثل تكون حصى الكلى.

***Keywords*:** Nanobacteria; Antibodies; Tissue Culture; Urolithiasis; Jordan

1. Introduction

Calcifying nanoparticles, or so called nanobacteria, were isolated and named by the Finnish researcher Olavi Kajander and the Turkish researcher Neva Ciftcioglu, working at the University of Kuopio in Finland (Kajander and Ciftcioglu, 1998). According to the researchers, the particles are self-replicated in microbiological culture, and the researchers further reported having identified DNA in these structures by staining (Carson, 1998). According to Kajander and Ciftcioglu, these are the smallest known self-replicating bacteria, and they are about 20–200 nm in length. CNPs are phylogenetically close relatives of mineral forming bacteria (Kajander et al., 1997; Kajander and Ciftcioglu, 1998). CNPs are thought to play an important role in extraskeletal calcifying diseases including periodontal stone formation, urolithiasis, atherosclerosis, chronic pancreatitis, rheumatoid arthritis, and various other tissues in the body (Carson, 1998). Apparently, these particles surround themselves with a mineral coating, and can serve as nidi for the genesis of renal calculi (Khullar et al., 2004; Ciftcioglu et al., 1999). Cuerpo (2000) showed that when these CNPs were injected intravenously, they accumulated in the kidney and produced apatite. The Finnish scientists

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who first discovered these CNPs have suggested that they are the *Helicobacter pylori* of kidney stone disease (Kajander et al., 1998; Ciftcioglu, et al., 1998).

Epidemiological studies have shown that only 10–20% of patients with renal stones have predisposing factors such as anatomical defects, metabolic or genetic disorders, and bowel disease (Parks and Coe, 1996). Others who develop stones due to any unknown cause are referred to as idiopathic stone formers. The progression of events leading to stone formation begins with urine supersaturation, crystal nucleation and aggregation, bringing about retention of crystals (nidi) and continued growth on the retained crystals (Menon and Resnick, 2002). Although the stimuli for calcium salt deposition are not completely known, it has become clear that nidi are needed for precipitation, even under supersaturated conditions.

CNPs antigens have been reported in 97% of human kidney stones (Kajander et al., 1997; Ciftcioglu et al., 1999). Apparently, these CNPs surround themselves with a mineral coating, and can serve as nidi for the genesis of renal calculi (Ciftcioglu et al., 1999; Cuerpo et al., 2000).

Urolithiasis in Jordanian population has increased in the last decade with high recurrence (Dajani et al. 1981; Rizvi et al. 2002). Local data indicates that urolithiasis in Jordanians may developed during their life without any known etiological factors (Freundlich et al. 1982; Dajani et al. 1988).

The present study is conducted to assess the incidence of the anti-CNP Abs in Jordanian patients, and to investigate the living nature of these CNPs by attempted isolation of them in cell co-culture from the collected renal stones.

2. Material and Methods

2.1. Patients and Samples Collection

Serum samples were collected from patients who were receiving medication at the Urology department, Islamic hospital, Amman, Jordan during the years 2006-2007. The healthy group included individuals undergoing routine checkup with no other known diseases. Individuals who were included in this study are from the same geographical area, all are resident in the central of Jordan, with close mean age and same risk and environmental factors (Table 1). Patients who had undergone operative procedures such as pyelolithotomy, extended pyelolithotomy and/or nephrolithotomy for the removal of renal stones were also included in the study. Surgically removed calculi from 20 patients with renal stones were collected. The stones were analyzed for their chemical composition by standard chemical analytical methods. (Abboud IA, 2008)

2.2. Enzyme Linked Immunosorbent Assay (ELISA)

Eighty five unrelated serum samples were collected, including 65 patients with urolithiasis [mean age (45±10)] and 20 healthy individuals [mean age (40±10) years] (Table 1). Diagnosis of urolithiasis was determined depending on the clinical presentation, physical examination, and also confirmed with radiological tests.

Duplicate of 100-µL diluted (1:500) serum samples were used to detect anti-CNP Abs with the Nano-Sero IgG ELISA kit (Nanobac OY, Finland). The absorbance was read at 450 nm, with the reference wavelength at 650 nm, using an ELISA reader (Sunrise Tecan, Austria). Anti-CNP Abs units were calculated from the standard curves using the kit standards via a linear equation. The assays were controlled using negative and positive controls. Anti-CNP Abs were classified as negative [unit value ≤2 × (mean of negative control - standard deviation)], borderline positive [unit value >2 × (mean of negative control - standard deviation), but <2 × (mean of negative control)] and positive [unit value ≥2 × (mean of negative control)] (Pretorius et al., 2004).

Table 1:Characteristics of the Study Population.

Variables	Study Participants	
	Patients Samples	Healthy Samples
Total number	65 (100.0)[a]	20 (100)
Gender		
Male	45 (69.2)	15 (75)
Female	20 (30.8)	5 (25)
Age (years)		
Mean	45±10	40±10
Range	20-75	20–70
Age groups (years)		
20–29	5 (7.7)	2 (10)
30–39	9 (13.8)	6 (30)
40–49	20 (30.8)	6 (30)
50-59	29 (44.6)	5 (25)
60 and over	2 (3.1)	1 (5)

[a] Numbers in parentheses are percentages.

2.3. Bacterial Cell Co-Culture

The stone samples were processed for the culture of CNPs according to Drancourt (2003), Khullar (2004) and Miller (2004). The stones were demineralised by incubation in 1 M HCl and neutralized with 1 M NaoH, (pH 10.5, Sigma) and the solutions were centrifuged at 20,000 *g* for 30 min at 4°C in a Sorvall RC5B centrifuge (GMI, Inc. USA). The pellet was suspended in serum free RPMI 1640 (Euroclone, Milan), filter sterilized through 0.2 µm Millipore filters (Sigma) and 1 ml of the filtrate were inoculated in screw-cap flasks containing 10ml of RPMI 1640 with 10% fetal bovine serum (FBS) (Gibco corp, USA). Cultures were incubated in CO_2 incubator (Heraeus, Germany) at 37°C, 5% CO_2 and 95% air for four weeks. Subcultures were carried out after 4 weeks of initial inoculation and subsequently after every 15 days. The cultures were aseptically precipitated by centrifugation at 20,000 g for 45 min at 4°C, washed with sterile phosphate buffered saline (PBS, pH 7.2), and then examined for bacterial growth by measuring the turbidity at 520 nm and 700 nm, using spectrophotometer (Hinotek, China) over a period of 6 weeks. RPMI with 10% FBS inoculated with no stone filtrate was used as a negative control. (Khullar, et al., 2004)

2.4. Bacterial Staining

Gram staining for the culture precipitant was done with a commercially available kit for any visible microorganisms (Wescor Inc., USA). Stained bacteria were observed under the oil immersion lens using the compound light microscope (Volker's optical, Germany).

Table 2. Anti CNP Antibodies in 65 Patients Samples.

Code	Antibody	Age	Sex	Code	Antibody	Age	Sex
1	0.36†	46	F	33	0.36†	55	M
2	0.56†	39	M	34	0.22*	56	M
3	1.37†	35	M	35	0.52†	56	F
4	0.80†	32	M	36	0.40†	51	M
5	0.75†	52	M	37	0.46†	52	M
6	0.59†	47	F	38	0.31†	50	M
7	0.38†	33	F	39	0.72†	52	M
8	0.29*	37	M	40	0.86†	53	M
9	0.64†	39	M	41	0.85†	55	M
10	3.38†	75	M	42	0.49†	51	M
11	0.66†	52	M	43	0.22*	53	M
12	0.29*	34	M	44	0.66†	54	M
13	0.62†	52	M	45	0.48†	59	M
14	0.73†	20	F	46	0.40†	56	M
15	0.36†	30	M	47	0.53†	53	M
16	0.49†	35	F	48	0.43†	22	M
17	0.40†	40	F	49	0.35†	46	M
18	0.41†	62	F	50	0.36†	45	M
19	0.42†	48	F	51	0.32†	47	M
20	0.15	23	F	52	0.50†	40	M
21	0.28*	53	F	53	0.56†	49	M
22	0.62†	55	M	54	0.85†	27	F
23	0.78†	40	M	55	0.82†	24	F
24	0.75†	52	M	56	0.33†	44	M
25	0.53†	58	M	57	0.56†	44	F
26	0.57†	51	M	58	0.19	45	M
27	0.83†	50	M	59	0.26*	58	F
28	0.82	43	M	60	0.27*	50	M
29	0.46†	46	F	61	0.85†	55	F
30	0.25*	47	M	62	0.32†	44	F
31	1.11†	55	M	63	0.25*	48	M
32	0.98†	47	F	64	0.69†	55	F
				65	0.24*	45	M

Antibodies were detected using enzyme-linked immunoassay (ELISA) kits and units were calculated from duplicate determinations. No symbol, negative results; †, positive results;*, borderline positive results; M, male; F, female.

Table 3. Anti CNP Antibodies in 20 Healthy Samples.

Code	Antibody	Age	Sex
1	0.13	21	M
2	0.40†	39	M
3	0.29*	22	M
4	1.42†	42	M
5	0.22*	40	M
6	0.16	52	M
7	0.38†	57	M
8	0.32†	36	M
9	0.13	43	M
10	0.08	51	M
11	0.11	33	M
12	0.12	40	M
13	0.15	34	M
14	0.18	42	M
15	0.12	38	M
16	0.32†	42	F
17	0.12	36	F
18	0.41†	55	F
19	0.16	51	F
20	0.19	70	F

Antibodies were detected using enzyme-linked immunoassay (ELISA) kits and units were calculated from duplicate determinations. No symbol, negative results; †, positive results;*, borderline positive results; M, male; F, female.

3. Results

3.1. Chemical Analysis of The Renal Stones

Renal stones that were included in this study were analyzed chemically by standard chemical analytical

methods. 12 stone samples out of 20 (60%) were calcium oxalate stones, and they formed the majority,6 (30%) were uric acid/urate, and only 2 (10%) were phosphatic.

3.2. CNP Antibodies

ELISA readings for the presence of anti-CNP Abs in the patients serum samples indicated that 53 out of 65 samples (81%) were positive, and 10 (15%) were border line, and 2 (3%) were negative (Table 2). For the healthy individuals 6 out of 20 samples (30%) had detectable anti-CNP Abs, 2 border line (10%), and 7 were negative (60%). (Table 3)

3.3. Bacterial Culture and Staining

Cell co-culture and Gram staining of inoculated RPMI 1640 medium showed no growth and detection of CNPs in any of the 20 stone samples and after one week of incubation. In addition, spectrophotometric of turbidity measurements for bacterial growth revealed the absence of any other microorganism in the tested culture media.

4. Discussion

Urolithiasis is a common disorder responsible for serious human suffering and economic cost to society. Approximately 16% of men and 7% of women in Jordan will be diagnosed with urolithiasis at some time in their life with a recurrence rate of more than 30% in 5 years (Personal communications and Bani Hani et al., 1998), this is compatible with other international data (Lloyd et al., 1996).

Even if some risk factors are defined for stone formation, none of them can fully explain the etiopathogenesis. In this study (data not shown), kidney stones were removed from patient less than 10 years of age, also other cases with urolithiasis form Jordanian children and teenagers are diagnosed and treated by surgical removal in spite of the ambiguous cause of such stone formation in their urinary tract (Dajani et al., 1988). Urolithiasis in young patients may be considered as a strong evidence of biological cause of stones formation.

The incidence of anti-CNP Abs was examined, and an attempt to culture these CNPs from Jordanian patients with urolithiasis was also done. Despite the fact of strict application of the methods described previously (Drancourt et al., 2003; Khullar et al., 2004; Miller et. al., 2004), cultures of CNPs were not obtained from kidney stones. The study therefore wonders if there is a culture parameters not mentioned in publications that could explain discrepancy between our results and those previously reported. However, a significant controversy has erupted over the existence and significance of CNPs as living or non living particles (Abbott, 1999; Abbott, 2000; Drancourt et al., 2003).

It has been suggested that biomineralization attributed to CNPs may be initiated by nonliving macromolecules like phospholipids and by self-propagating microcrystalline apatite (Cisar et al., 2000). Alternatively, the formal possibility exists that our specimens simply did not contain any living substances, and so the living nature of the CNPs is remain unresolved. Conflicting results have been reported concerning the bacterial culture succeed, Khullar (2004) successfully cultured 40 different renal stones from patients with nephrolithiasis. In contrast, Drancourt (2003) failed to culture CNPs from 10 upper urinary tract stones.

High anti-NB Abs distribution in both patients and healthy study groups proved high rate of CNPs exposure. CNPs may found in different samples like environmental and animal samples, and may be transmitted directly to human beings. (Kajander and Ciftcioglu, 1998; Travis, 1998). Other studies suggest that transplacental or perinatal transmission of CNPs and anti-NB Abs from infected mothers to their babies could be possible (Pretorius et al., 2004).

High detection of anti-CNP Abs in Jordanian population (96% of patients and in 40% of healthy individuals) was correlated positively with urolithiasis. In addition, anti-CNP Abs level was inversely correlated with the severity and the recurrence of many other extraskeletal diseases (Kajander and Ciftcioglu, 1998). CNPs as a cause of urolithiasis are not assessed in Jordanian hospitals. To the best knowledge of the researcher, this is the first observational clinical study to demonstrate the incidence and to distribute anti-CNPs Abs in Jordanian population with urolithiasis.

The scale of the urolithiasis epidemic in Jordan provides many opportunities to study the impact of the

etiology. The source of CNPs acquisition and the mode of transmission of such particles are still unknown. The environmental source is the most possible rout of obtaining CNPs in Jordanian patients, and must be studied further.

Further tests like electronic microscopy, histochemistry staining, and PCR analysis must be performed for CNPs co-culture plates in order to confirm the presence or absence of any bacterial growth.

Results presented in this pilot study could have therapeutic relevance and links between CNPs and urolithiasis, especially in individuals with family history for kidney stones formation.

Further studies are required to validate the living nature of CNPs, to establish the exact mechanism by which CNPs are involved in the causation of renal stones, and to assess the role of the anti-CNP Abs distribution as a prediction of any extraskeletal calcification.

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Variations of Heavy Metals Concentration in Suspended Matter and Physiochemical Properties in the Coastal Surface Water of the Gulf of Aqaba.

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Abstract

Variations of heavy metals concentrations (Pb, Zn, Ni and Cd) in suspended matter as well as the physical properties (temperature, salinity, dissolved oxygen and pH) at five coastal sites and one reference station (offshore) were measured on seasonal base of the year 2005. Seasonal variations of seawater temperature, salinity, pH and dissolved oxygen were significant different, meanwhile; spatial variations among different stations were not significant. Temporal variations of heavy metal concentrations were significantly different (P< 0.001); the highest concentrations were detected in spring 0.003 and 0.137 ppm for Pb and Zn respectively. Very low concentrations of Cd (not exceed 0.001 ppm) were detected in autumn and winter. Very low concentrations of Pb, Zn and Cd metals were detected in all sampling stations except Ni the concentrations were ranged between 0.12 and 0.108 ppm; however, variations among different sampling sites were not significant (p= 0.84)

الملخص

لقد تم دراسة التغير في تراكيز العناصر النزرة (Pb, Zn, Ni, Cd) في المواد العالقة و كذالك الخصائص الفيزيا ئية للمياه (درجة الحرارة، الملوحة، الاكسجين الذائب و درجة الملوحة) في خمس مناطق ساحلية و منطقة واحدة مرجعية (المياه المفتوحة) على اساس فصيلي للعام 2005 , لقد اضهرت نتائج الدراسة تغيرات معنويةفي درجة الحرارة ، الملوحة، الحموضة و الاكسجين المذاب للفصول المختلفة بينما لم تظهر اي تغيرات جوهرية بين الاماكن المختلفة. التغير الزماني لتراكيز العناصر المختلفة كان جوهريا (P< 0.001) بينما التغير المكاني لتراكيز العناصر المختلفة لم يكن جوهريا (P= 0.84).اعلى تركيز تم رصده خلال فصل الربيع 0.137 جزوء في المليون لعنصر Zn و 0.003 جزوء في المليون لعنصر Pb, اقل تركيز تم رصده خلال فصلا ا لخريف والشتاء لعنصر Cd (0.001 جزوء في المليون).لقد اظهرت الدراسة تراكيز قليلة للعناصر (Cd, Zn, Pb) في جميع مناطق الدراسة ما عدا عنصر Ni فقد تم رصد تراكيز تراوحت ما بين 0.12 -0.108 جزوء في المليون.

Keywords: Physiochemical Properties; Heavy Metals, Suspended Matter; Gulf of Aqaba; Red Sea

1. Introduction

Considerable evidence in the scientific literature that contaminants such as trace metals can be taken up and concentrated by sediments and suspended matter in the Aquatic systems (Forstner and Wittman, 1979; Hart, 1982). Transportation of theses contaminants in association with particulate matter represents a major pathway in the biogeochemical cycling of trace contaminants (Hart, 1982).

The vertical and horizontal distributions of many trace elements in the ocean are determined by association with the cycle of growth, sinking and demineralization of marine phytoplankton. Phytoplankton in the oligotrophic northern Red Sea and Gulf of Aqaba is characterized by a low biomass (<0.8 mg chlorophyll l^{-1} of seawater), in which the water ware dominated by prochlorophytes during early summer and fall, meanwhile, during winter Eukaryotic algae were dominate (Sommer *et al.,* 2002; Al-Najjar, 2000). Phytoplankton, which serves as food for herbivorous fishes and higher organisms, is found to absorb significant amounts of dissolved organic matter from seawater. Hence, phytoplankton is found to be highly susceptible to various contaminants, such as, hydrocarbon, crude oil, metals and industrial effluents (Cushing and Walsh, 1976). Several Authors (Martin *et al.,* 1990; Grotti *et al.,* 2001)

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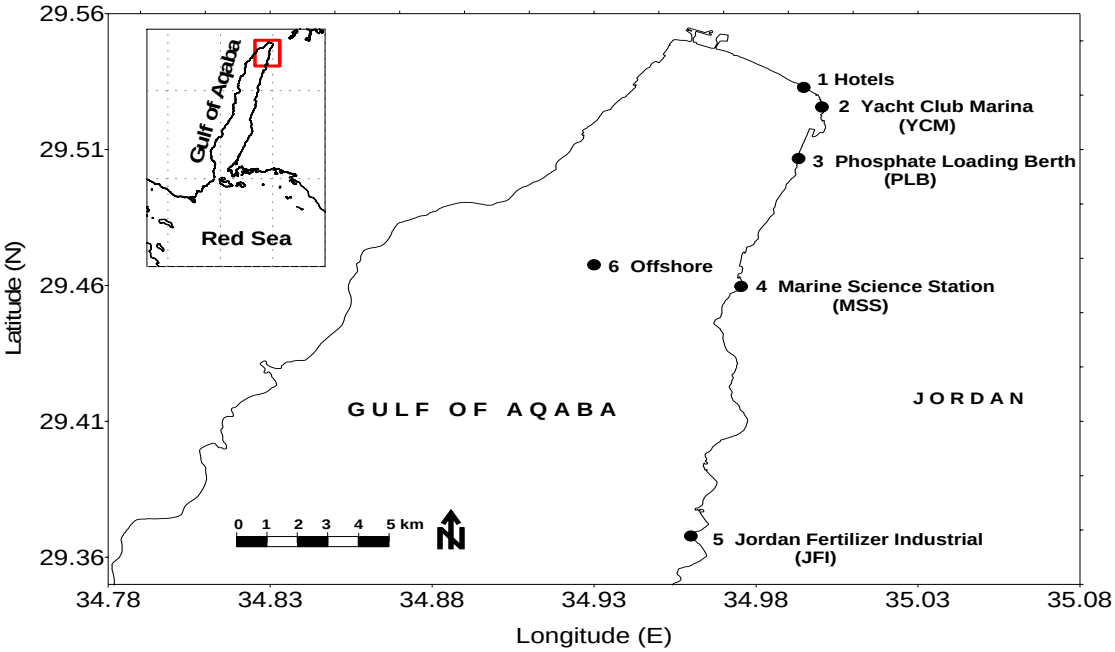

Figure 1. Study area and sampling sites in the northern Gulf of Aqaba.

Reported that the suspended matter of the coastal waters characteristically have much higher concentrations of trace metals such as Fe, Mn, Co, Cu. Cd and Zn than offshore waters, probably due to the input of atmospheric dust from land, nutrient availability, and plankton ecology. Physical and biological processes in the coastal areas have been reported to affect the concentration of their trace metals (Martin *et al.,* 1990; Sedwick *et al.,* 1997; Sohrin *et al.,* 2000).

No information is available on the levels of heavy metal in suspended matter of the coastal areas of the Gulf of Aqaba, which is subjected to different types of human activities, development and uses. The aim of this study is to fill a gap in the information on the levels of Cd, Ni, Pb, and Zn in suspended matter of the five coastal stations and one offshore station in the Gulf of Aqaba.

2. Materials and methods

2.1. Study Area

The Gulf of Aqaba is a partially enclosed water body that constitutes the eastern segment of the V-shaped northern extinction of the Red Sea (Figure 1). It is located in a sub tropical arid area between 28º29′-56º north and 34°30′-35° east. The Gulf of Aqaba is 180 km long and has a maximum width of 25 km, which decreases at the northern tip to about 5 km. It is connected to the Red Sea through the Strait of Tiran, which has a depth of about 252 m (Hall, 1975; Por and Lerner-Seggev, 1966). The present study area lies within the Jordanian portion of the Gulf of Aqaba, which extended for about 27 Km to the north of the border with Saudi Arabia, and constitutes the most northern and northeastern side of the Gulf.

2.2. Temperature, Salinity, Ph and Dissolved Oxygen Measurements

Temperature, salinity, pH and dissolved oxygen were measured seasonally (winter, spring, summer and autumn, 2005) down to 30 m depth from five coastal sites; Hotels, Royal Yacht Club (YCM), Phosphate Loading Berth (PLB), Marine Science Station (MSS) and Industrial Complex/Jordan Fertilizer Industry (JFI), and one Reference station (Offshore) (Figure 1), using Conductivity, temperature and depth meter (OC 7316-Idronayt, CTD). These sites embrace various habitats such as fringing coral reef, seagrass beds and unconsolidated sandy bottom areas. In addition, the selected sites represent portions of the coastal zone where major development, and maritime, industry and tourism activities are taking place.

2.3. Suspended Matter Collection and Treatment

Suspended matters were collected on a seasonal base of the year 2005 from the five coastal sites, and one offshore station. Two liters of seawater were sampled using 5 liter Niskin bottle; samples were put in a precleaned plastic bottles with 1% HCL, and brought to the laboratory immediately collection, where each sample was filtered on a GFC filter paper 0.45µm. Filters were then dried at 85^{o}C to constant weight for about 24 hrs, and stored in a plastic bag for future trace metal analysis.

2.4. Samples Digestion and Heavy Metals Measurement

The filters were placed in pre-cleaned small capacity (100 ml) glass beakers, and oxidized by the addition of 8ml of 69.5% ultra-pure nitric acid at room temperature for 4 hrs. Beakers were put on a hot plate at 100^{o}C for 6 hrs, and then allowed to cool to room temperature. The samples were heated again to near dryness in order to remove the nitric acid. The residue was dissolved in 8ml of 1% nitric acid and kept on a hot plate for about 1 hr to enhance dissolution. The samples were allowed to cool to room temperature and then filtered on a Whatman filter paper

number 43. Samples were finally diluted to 25ml with 1% nitric acid. Concentrations of Mg, Cd, Cu, Ni, Pb, Fe and Zn were measured by the use of Jena AA 300 atomic absorption spectrophotometer. Duplicate measurements were made for each sample, by direct aspiration into air-acetylene flame. The instrument was instructed to give the mean value and standard deviations of three readings as the final reading of each sample. The precision of the whole procedure was assessed by 10 replicates for a sample and the results agreed to within 3%. Duplicate blanks were used for each batch of digested samples. The mean value of the blank was subtracted from the readings of the sample to give the final reading. In addition to the blank solution, three standard solutions were prepared to cover the expected range of the element concentrations in the samples and within the linearity of the procedure (within the linear portion of the calibration curve of the procedure). The final element concentrations were interpolated as ppm unit.

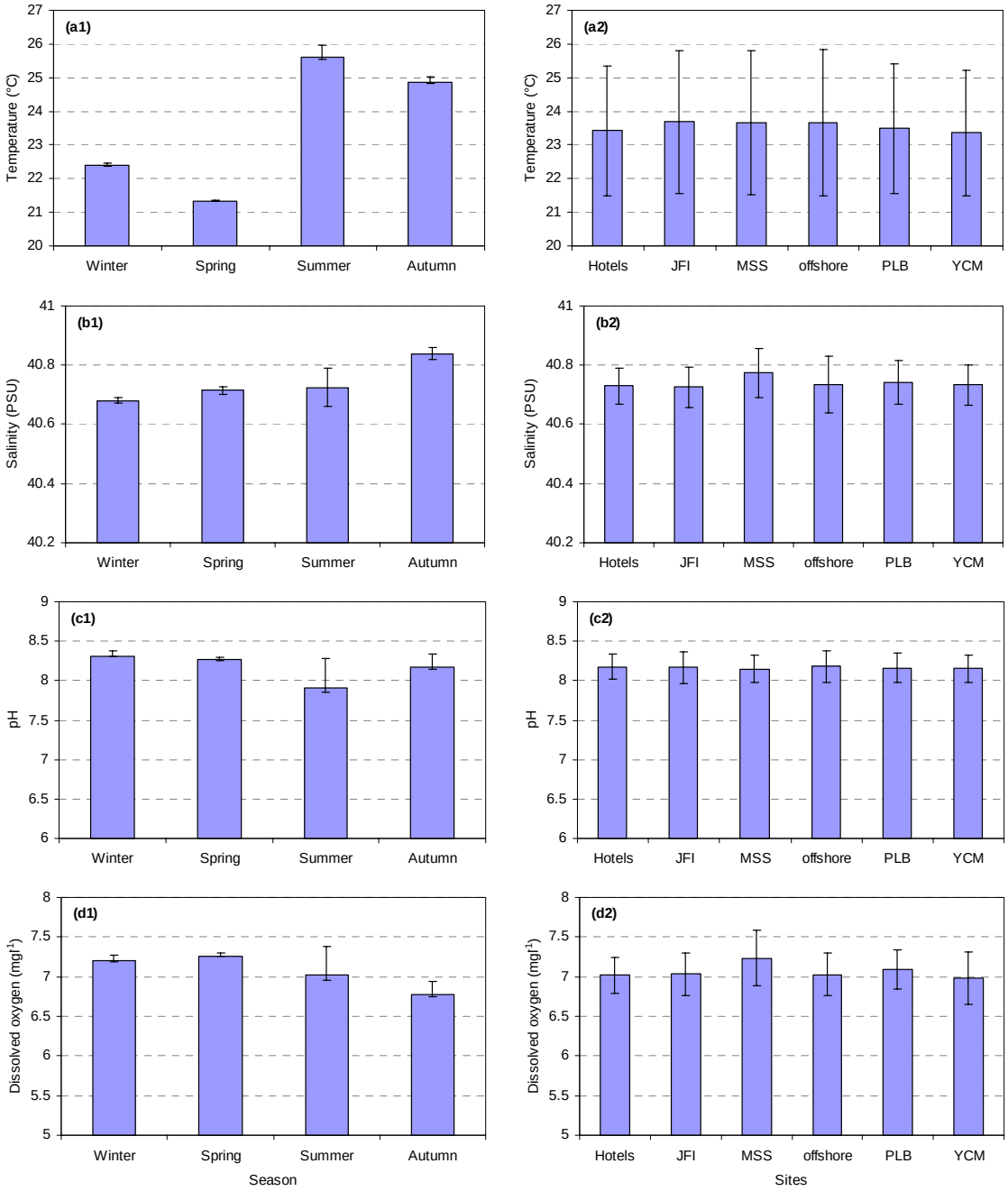

Figure 2. Spatial and temporal variations of (a) temperature (°C), (b) salinity (PSU), (c) pH and (d) dissolved oxygen (mgl^{-1}) of water column along the study area in the northern Gulf of Aqaba, Red Sea. Standard deviation are the barrs ?.

3. Results

3.1. Water Temperature, Salinity, Ph and Dissolved Oxygen

Seasonal variations of seawater temperature were significant (= 0.0001).The highest mean value (25.61 ^{o}C) was recorded in summer, whereas the lowest mean (21.33 ^{o}C) was recorded in spring. Spatial variations among different stations were not significant (p= 0.90), where the mean temperature values ranged between 23.36 ^{o}C at YCM and 23.68 ^{o}C at JFI (Figure 2. a).

Temporal variations of seawater salinity were significant (p= 0.001). The highest mean salinity (40.84 PSU) was recorded in autumn, whereas the lowest mean (40.68 PSU) was recorded in winter. In comparison, spatial variations in salinity between different stations were not significant (p= 0.95), whereas the mean value was 40.73 PSU at JFI and 40.77 PSU at MSS (Figure 2b).

Similarly, temporal variations in pH were also significant (p< 0.0001).with the highest mean value (8.32) was winter, while, the lowest in mean value (7.91) occurred in summer. Variations of pH among the sampling

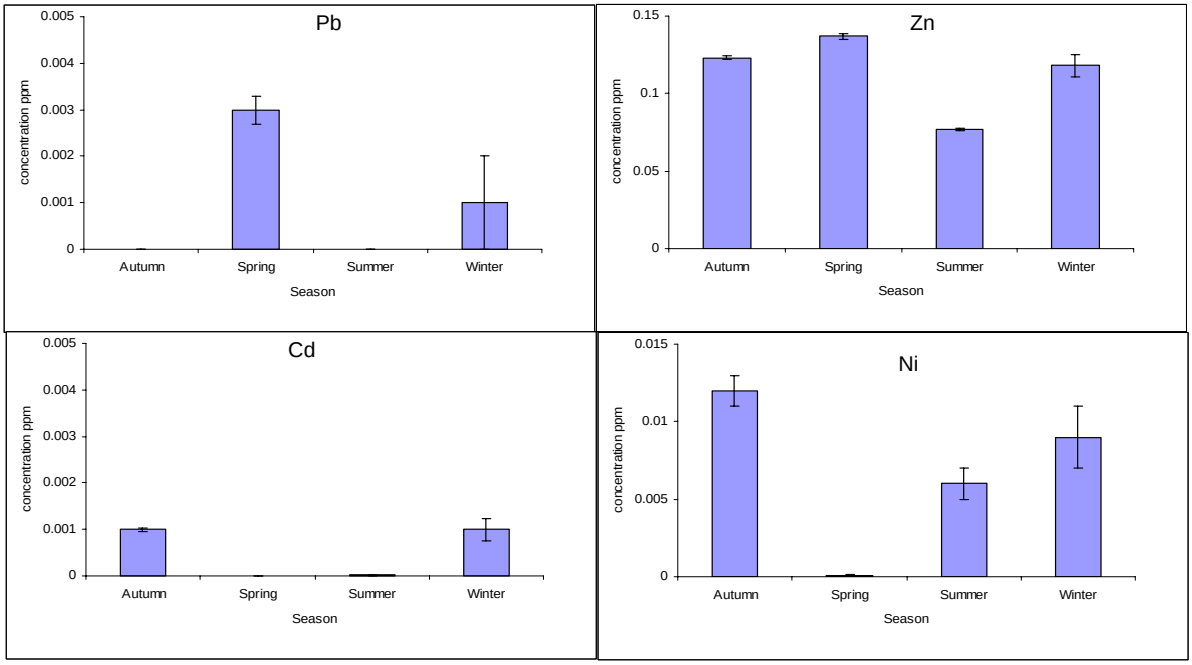

Figure 3. Temporal mean concentrations ± SE of Pb, Zn, Ni, and Cd in phytoplankton from the Jordanian coast of the Gulf of Aqaba, Red Sea.

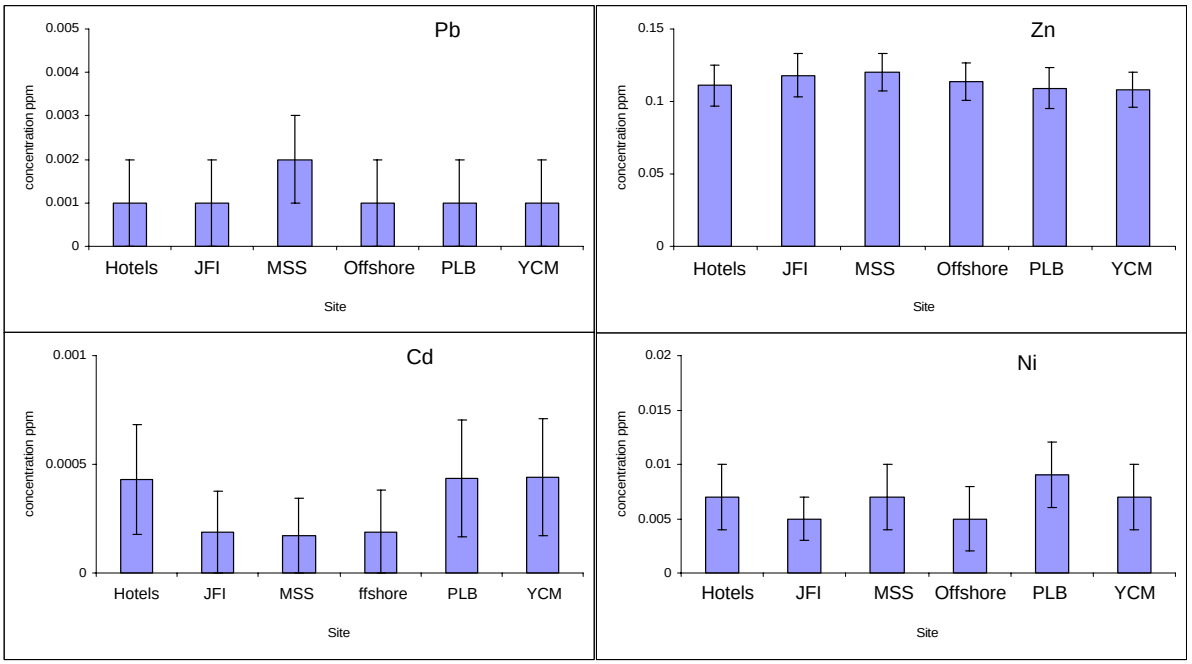

Figure 4. Spatial mean concentrations ±SE of Pb, Zn, Ni, and Cd in phytoplankton from the Jordanian coast of the Gulf of Aqaba, Red Sea.

sites were not significant (p= 0.99), where the mean pH value ranged between 8.15 at MSS and 8.18 at Offshore (Figure 2c).

Temporal variations (p= 0.0013) in dissolved oxygen occurred during sampling dates; highest value was recorded in spring with mean value of 7.26 mg. l^{-1}, whereas the lowest value was in autumn with mean value of 6.77mg. l^{-1}. Spatial variations of dissolved oxygen were not significant (p= 0.833), the mean value was 7.24 mg. l^{-1} at MSS and 6.98 mg. l^{-1} at YCM (Figure 2d).

3.2. Heavy Metals in Suspended Matter

3.2.1. Temporal Variations

The mean concentration of Pb for spring and winter were 0.003 ppm and 0.001 ppm, respectively. However, no Pb was detected in autumn and summer. The season highest Zn concentration (0.137 ppm) occurred in spring, compared to a concentration of 0.077 ppm which was found in summer. The highest Ni concentration was obtained in autumn (0.01 ppm) while, lowest concentrations 0.0001 ppm were obtained in spring. The Cd concentrations did not exceed 0.001 ppm in both winter and autumn, while no Cd was detected in summer and spring. However the one way ANOVA test showed significant temporal variations between different seasons (P<0.001) (figure. 3).

3.2.2. Spatial Variations

The Pb concentrations at all sites were in the range of 0.001- 0.002 ppm. Concentrations of Zn in all sampling sites ranged between 0.12 ppm at the MSS and 0.108 ppm at the Yacht Club site. Ni concentrations were low at all sampling sites and ranged between 0.005 ppm at the JFI and 0.009 ppm at offshore, and Phosphate sampling sites. Very low concentrations of Cd were measured at all sampling sites, where it ranged between 0.00017 ppm at the MSS and 0.0004 ppm at the Yacht Club sampling site. The statistical test using one way ANOVA showed no significant differences among different sampling sites (P= 0.84) (Figure. 4).

4. Discussion

4.1. Physical Conditions

The main temperature differences were recorded at the surface with mean values of 25.61 ^{o}C in summer and 21.33 ^{o}C in spring. The interannual variation of surface temperature is mainly linked to variation in the net heat flux (Genin *et al.,* 1995). The main trend of variation in the present study is in a good agreement with those reported in previous studies from the Gulf of Aqaba. Paldor and Anati (1979) reported a maximum surface water temperature of 26.7 ^{o}C in July and a minimum of 20.8 ^{o}C in March. Badran (1996) found a maximum surface water temperature 27.5^{o}C and a minimum of 20.8 ^{o}C. Manasrah (1998) reported a maximum surface water temperature of 25.48 ^{o}C in August and a minimum of 21.25 ^{o}C in April which is in agreement with the results obtained in the previous study. Moreover, Manasrah and Badran (2008, in press) found that the minimum and maximum surface temperature was 21.16 in February 2001 and 27.99 in August 1999.

In the present investigation, the salinity of surface water varied between 40.84 PSU in autumn and 40.68 PSU in winter. The temporal and spatial variations of surface salinity were very small and not significant compare the high value of salinity itself. Al-Najjar (2000) found that very small variations in surface water salinity, which ranged from 40.62 PSU in September to 40.33 PSU in April. Manasrah and Badran (2008, in press) reported that the minimum and maximum surface salinity value during 1997-2003 was 40.2 PSU and 40.7 PSU, respectively.

4.2. Spatial and Temporal Variability in Metal Concentrations in Suspended Matter

A considerable amount of trace metal has been concentrated by suspended matter in different locations along the Jordanian coast of the Gulf of Aqaba. The suspended matter contents of Zn are relatively high at the Marine Science Station. By comparison, the suspended water samples from the Yacht club and phosphate loading port showed apparently high concentration of Cd, Mg and Fe. Those from Phosphate Loading Port showed apparent high concentration of Fe and Ni. However, the statistical examination did not show any significant differences among the six sampling sites. The higher concentrations of suspended metals in the Marine Science Station can be explained in view that the site is exposed to the effects of different anthropogenic activities on the passenger port (Abu-Hilal and Al-Najjar, 2004). The high concentration of Cd in Yacht Club is mainly due to the relatively high activity of boats and their maintenance which includes painting and cleaning. The present results are in general agreement with the results of previous works (Al-Batainih, 2004; Ababneh, 2004) on bioaccumulation of trace metals in the tissue of bivalves (*Modiolus auriculatus*) at different locations along the Jordanian coast. However, in an overall view of the study have been made by Bu-Olayan *et al.,* 2001 concerning the distribution of different trace metals in different sites along the coast of Kuwait supporting our finding in which the elevation of some trace metals have been attribute to the input of metals from dust and industry, associated with pollution activities were found susceptible to environmental hazards in the marine ecosystem and mankind. Examination of the results of the present study showed that the phytoplankton metal concentrations was in the following order Zn> Ni> Pb> Cd. It is obvious that the Zn occurred in highest concentration. This is to be expected and could be explained in the view that this element is considered as essential trace element for cell growth and differentiation in several species of marine organisms such as an integral part of respiratory protein, and required for the activity of diverse enzymes and the healthy living of plankton organisms (Bryan, 1968; Ghidalia, 1995; Aaseth and Norseth, 1986; Adams *et al.,* 1982; Senkebeil and Wriston, 1981; Toma, 1984; Gherardi, *et al.,* 2001). However, it is known that at elevated concentrations these metals become toxic to organisms (Phillips, 1980). By comparison, the concentrations of the non essential highly toxic metals such as Cd and Pb are the lowest in availability (Gouvea *et al.,* 2005).

The careful examination of the result indicate that seasonal variation of metal in suspended particles have been observed in this study for all measured elements

where higher concentrations were detected mainly during autumn and spring. This variation could be related to the changes in the pollution load of the studies sites as suggested by Ritterhoff and Zauke (1997).

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Assessment of Genetic Diversity Among Wheat Varieties in Sulaimanyah using Random Amplified Polymorphic DNA (RAPD) Analysis

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Abstract

Genetic diversity among 11 durum and bread wheat genotypes was studied using random amplified polymorphic DNA (RAPD) analysis. A total of 70-75 DNA fragments were amplified with 10 random decamer primers 40% (bread , and 35.7% (durum wheat) of which were polymorphic. Genetic similarity matrix based on Dice index detected coefficients ranging from 0.5 to 0.952 (bread wheat) and 0.102 to 0.917 (durum wheat). These coefficients were used to construct a dendrogram using unweighted pair group of arithmetic means (UPGMA). The bread wheat genotypes were clustered into two major groups; first group includes Cham 4 and Cham 6; and the second includes Tammuz, Aras, and Rabia. The durum wheat genotypes were classified into two groups: group 1 presents the varieties Acsad 65 and Cemmitto, and group 2 contains Creso Kurde, Creso Italy, Ovanto, and Bakrajo 1. The highest similarity among the wheat varieties was observed between Cham 4 and Cham 6 for bread wheat and Bakrajo 1 and Ovanto for the durum wheat. The most distant genotype in the dendrogram was Acsad 65 and Aras. It has been clearly shown that most of the varieties possessed narrow genetic background. The information would be helpful for future genome mapping programs as well as for the application of intellectual breeder rights. The study will also work as indicator for wheat breeders to evolve varieties with diverse genetic background to achieve sustainability in wheat production in the country

الملخص

تمت دراست الاختلاف الوراثى بين اصناف من الحنطة الناعمة والخشنة باستخدام التضخيم العشوائي للتعدد الأشكال الحمض النووي الريبي منقوص الأكسجين. ووجدت 70-75 قطعة من الحمض النووي الريبي منقوص الأكسجين،كما وجدت الاختلاف الوراثى بنسبة 40% فى الحنطة الناعمة و 37.5% فى حنطة الخشنة. وان التشابه الوراثى تتراوح بين 0.5-0.952 فى الحنطة الناعمة و 0.102-0.917 فى الحنطة الخشنة. وتم استخدام التشابة الوراثى لتكوين شجرة التنوع الوراثى. وباسناد على نتائج الشجرة التنوع الوراثى، قسمت الحنطة الناعمة الى مجموعتين رئيسيتين : المجموعة الاولى يشمل شام 4 وشام 6، والثانية تشمل تموز،أراس وربيعة. و صنفت الحنطة الخشنة الى مجموعتين: المجموعة الاولى يشمل اكساد 65 و سيميتو والثانية تشمل كريزو كردى، كريزو ايطالى، اوفانتو وبكرجو. كما لوحظت اعلى نسبة التشابة بين الصنفين شام 4 وشام 6 فى الحنطة الناعمة والصنفين اوفانتو و بكرجو فى الحنطة الخشنة. واظهرت اعلى نسبة من الاختلاف الوراثى فى الاصناف أراس و اكساد 65مقارنة بالاصناف الاخرى. ولقد ثبت بوضوح أن معظم الاصناف يمتلك الخلفية الضيقة. وان نتائج هذة الدراسة سوف تكون مفيدة فى المجال تكوين الخرائط الكروموسومية والبرامج التربية و التحسين فى العراق.

Key words: Wheat; DNA; RAPD; Genetic Diversity

1. Introduction

Wheat is a staple food; and is one of the important agricultural crops, which is a basis for human nutrition; and is of enormous economic importance to both Iraq and worldwide. Historically, many genetic markers such as morphological markers (Porter and Smith, 1982) and bio-chemical markers such as isozymes and seed storage proteins (Miller et al., 1989) have been used to monitor and maintain germplasm biodiversity. These markers were more prone to environmental effect and limited by small number of loci (Tanksley, 1983; Tanksley et al., 1989). DNA markers have facilitated genetic studies in plant, animal, and prokaryotic genomes (Mullis, 1990; Erlich et al., 1991). Among the several DNA based techniques, random amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams et al., 1990) gained importance due to its simplicity, efficiency, and non

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requirement of sequence information (Gepts, 1993; Karp et al., 1997).

RAPD provides virtually limitless set of descriptors to compare individual plants and the population. With this innovative tool, genetic diversity can be estimated (Demeke et al., 1996), and equally it is possible to carry out large scale screening of genetic resources held in gene banks, natural populations, ecosystems, and natural reserves with this quick and rapid technique. Analysis of RAPD is based on the amplification of DNA fragments with the polymerase chain reaction (PCR) starting from primers with arbitrary sequences (Williams et al., 1990). This technique is considerably faster and simpler than some other molecular techniques. RAPD markers have been used to examine both interspecific and intraspecific variations in a number of plant species (Kazan et al., 1993; Bai et al., 1998). The analysis of SSR, based on the PCR, is also easier to perform than other molecular analysis, and is highly amenable to automation. RAPD analysis has been extensively used to document genetic variation in *Triticum* (Cao et al., 1998; Sun et al., 1998; Bedo et al., 2000; Czaplicki et al., 2000; Gerashchenkov et al., 2000; Gupta et al., 2000; Yuejin and Lin, 2000), suggesting a narrow genetic base. RAPD markers have also been used for cultivar identification (Hu and Quirose, 1991; Malik et al., 1996), fingerprinting of genomes (Nybom et al., 1989, Welsh and McClelland, 1990), and for tagging of genes (Klein-Lankhorst et al., 1991; Martin et al., 1991; Rafalski et al., 1991; Kelly et al., 1993). The objective of this study was to analyze RAPD-based genetic variance among 11 varieties of wheat.

Table 1. Nucleotide sequences of the 10 primers used in this study (Naghavi, 2004).

Primer name	Sequence 5 ⟶ 3
UBC1	CCTGGGCTTC
UBC3	CCTGGGCTTA
UBC9	CCTGCGCTTA
UBC13	CCTGGGTGGA
UBC104	GGGCAATGAT
UBC105	CTCGGGTGGG
UBC106	CGTCTGCCCG
UBC108	GTATTGCCCT
UBC109	TGTACGTGAC
UBC110	TAGCCCGCTT

2. Materials and Methods

2.1. Plant Materiel and DNA Extraction

Seeds of 5 bread wheat varieties and 6 durum wheat varieties were obtained from the Department of Agriculture in Sulaimanyah. DNA was isolated from bulks containing equal quantities of leaf tissue from 10 plants. Leaves were collected and frozen in liquid nitrogen, and then crushed to make a fine powder. One hundred milligram of fine powder was used for DNA extraction. Five hundred micro-liter of CTAB buffer (1.4 M NaCl,

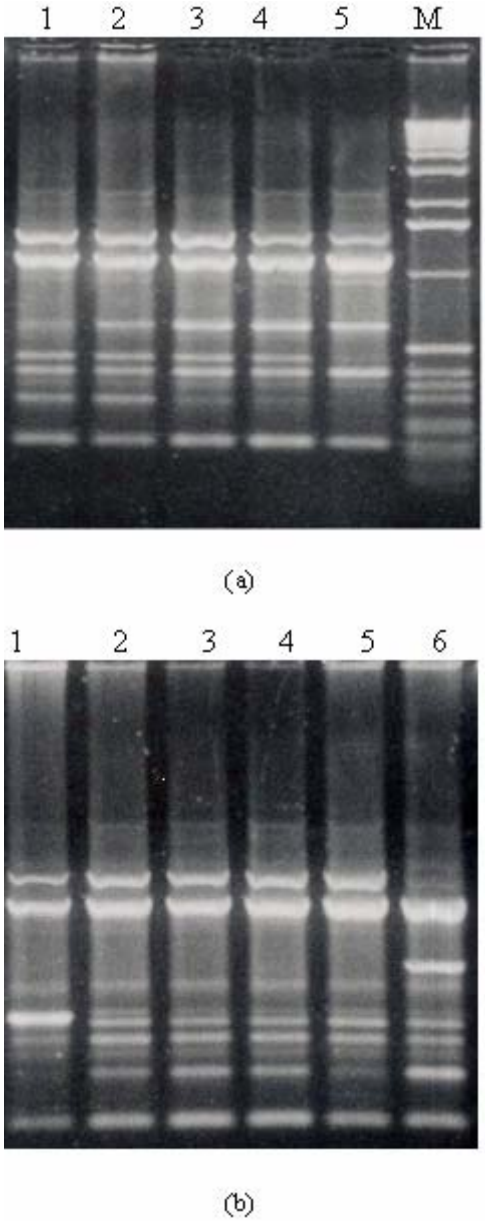


Figure 1: An example of RAPD banding pattern obtained from primer UBC3 on 11 genotypes of wheat; **a.** Bread wheat : 1= Tammuz, 2= Rabia, 3=Aras, 4=Cham 6, 5=Cham 4, M: marker size; **b.** Durum wheat: 1=Acsad 65, 2=Bakrajo 1, 3=Cemmitto, 4=Creso Kurde, 5= Ovanto, 6= Creso Italy.

100 mM Tris, 20 mM EDTA, pH 8, 2% CTAB) was added to each eppendorf tube containing the crushed leaf material; and was thoroughly mixed by pipetting. The mix was incubated for 60 min at 60°C. Equal volume (500 µl) of chloroform: isoamyl alcohol (24:1) was added, and tubes were then shaken until a homogenous mixture was obtained. Samples were then centrifuged at 10000 rpm for 7 minutes in a bench centrifuge. The aqueous phase was transferred to a fresh tube. Ammonium acetate [0.08 volume of cold 7.5 M (32 µl)] and cold isopropanol [0.54 volumes of (233 µl)] were added in the tube and were mixed gently to precipitate the DNA at -80 °C for one hour. Samples were centrifuged at 10000 rpm for 7 minutes to pellet the DNA. After discarding the supernatant, the pellet was washed three times with 70%

ethanol. Pellet was dried at room temperature for one hour and re-suspended in 40 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). To remove RNA, DNA was treated with 40 µg RNAse-A at 37°C for one hour and was stored at 4°C until used. To use in PCR, 1:5 dilution of DNA was made in double distilled deionized and autoclaved water.

Table 2. Effectiveness of RAPD marker in detecting polymorphism of wheat varieties.

	Bread wheat	Durum wheat
Number of assay units (primer)	10	10
Total bands scored	75	70
Polymorphic fragment scored	30	25
Percentage of polymorphism	40	35.71
Minimum polymorphism scored per pair primers	1	1
Maximum polymorphism scored per pair primers	4	5
Average polymorphism scored per pair primer	3	2.5

2.2. PCR (Polymerase Chain Reaction) Amplification

The amplification reaction contained 1XPCR mix, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 50 ng of each primer, 1 U Taq polymerase and 20 ng template DNA. Amplifications were performed in a Biometra gradient thermocycler with the following cycling profile: an initial denaturation at 92°C for 5 min, followed by 40 cycles of 1 min at 92°C, 1 min at 37°C and 2 min at 72°C with a final extension for 6 min at 72°C. PCR products were mixed with 1/5 vol of loading buffer and separated on a 2% (w/v) agarose gel in 1X TBE at 70 V for 3 h. The gels were then stained in a 0.4 μg/mL ethidiumbromide bath and the DNA fragments visualized under UV light.

2.3. DATA Analysis

The data obtained with the technique RAPD w scored in a binary form as the presence or absence (1/0) of bands for each sample. SPSS was used to calculate DICE similarity coefficient. The similarity matrices were converted into distances matrices; and used to generate dendrograms by UPGMA.

3. Results and Discussion

In order to increase the confidence level of the fragments included in the matrices (for RAPD), Using this approach, it is possible to lose more than one useful information, but the aim was to obtain reproducible and clear data.

Electrophoresis of PCR products on 2% agarose gels containing ethidium bromide (Figure 1A and B) revealed different degrees of polymorphism for different primers (Table 3). In the RAPD analysis, ten-mer primers were used to amplify all of the genotypes, ll primers showed reproducible and well-resolved bands. These primers produced fragments ranging from about 400 bp to 3,500 bp in size. A total of 70-75 fragments were observed from these primers. In total, 30 polymorphic bands for bread wheat and 25 polymorphic bands for durum wheat were detected. The highest number of polymorphic and scorable bands was obtained by primer UBC13, the lowest by primers UBC105, and UBC106 (Table 2).

The RAPDs generated were used to determine the genetic distances between the wheat varieties. The relationship of these varieties, as identified by the classification, has been represented as a dendrogram (Figures 2 and 3).

The genetic similarity for pairs of species was calculated using Dice coefficients (Tables 3 and 4). The similarity matrix based on all possible pairs of varieites ranged from 0.105 to 0.917 for bread wheat and 0.5 to 0.952 for durum wheat (Tables 3 and 4). The lowest pair-wise similarity matrix value was between Tammuz and Cham 4 (0.105) for bread wheat and between Acsad 65 with Italy and Cemmitto (0.5). This reveals a relatively high degree of genetic variability within the species. The highest pair-wise similarity was between Cham 6 and Cham 4 (0.917) for bread wheat and between Bakrajo 1 and Ovanto (0.952) for durum wheat. The reason for this higher similarity was that two varieties have the same parents.

The dendrograms were constructed to express the similarity among the varieties based on the RAPD (Figure 2). Cluster analysis was carried out by the UPGMA method on the Dice similarity coefficients. The position of the genotypes in different clusters is presented in Figure 2. The dendrogram constructed with UPGMA revealed that 11 genotypes fell into different distinct groups. The dendrogram divided the bread wheat varieties into two groups: group 1 includes Tammuz, Aras, and Rabia while group 2 includes Cham 4 and Cham 6. The varieties Cham 4 and Cham 6 showed high similarity (Figure 2). The most distant genotype in the dendrogram was Aras. On the other hand, the dendrogram revealed two groups for durum wheat. group 1 contains the Creso Kurde, Creso Italy, Ovanto and Bakrajo 1. The second group includes Acsad 65 and Cemmitto (Figure 3). The varities Ovanto and Bakrajo 1 showed high similarity, and the variety Acsad 65 revealed the highest distance.

Table 3. Similarity matrix showing the relationship among the bread wheat genotypes based on RAPD data.

Varieties	Tammuz	Rabia	Aras	Cham 6	Cham 4
Tammuz	1,000				
Rabia	0,625	1,000			
Aras	0,421	0,667	1,000		
Cham 6	0,211	0,476	0,500	1,000	
Cham 4	0,105	0,381	0,417	0,917	1,000

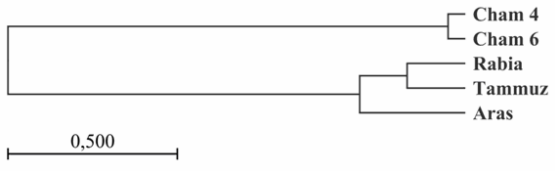


Figure 2. Dendrogram of bread wheat varieties showing genetic similarity based on RAPD data by using UPGMA cluster analysis.

In this study, the polymerase chain reaction (PCR)-based systems (RAPD) have been used and compared for studding the genetic diversity between 11 cultivars of

wheat., RAPD analysis was found to be a valuable diagnostic DNA marker system for evaluating genetic diversity. The information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform and endangering long term productivity gains (Messmer et al., 1992). The number of primers used in the RAPD method should be neither too small, because this could lead to a noninformative or biased analysis, nor too high, which could result in increased cost.

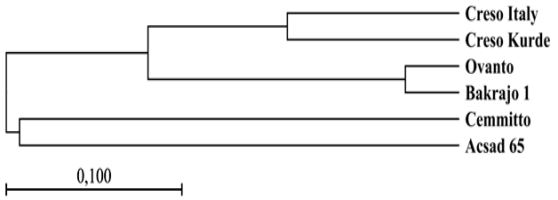
The level of polymorphism for bread wheat (40%) was superior to the level of polymorphism for durum wheat (35%). In this study RAPD markers were able to discriminate Cham 4 and Cham 6, and also this technique able to find some polymorphisms between the Creso Kurde and Creso Italy. This technique showed highest variability between the varieties of bread wheat with comparing with the varieties of durum wheat. This less similarity among the varieties may be attributable to the difference of center, which developed these varieties or a high degree of genetic differences may be the usage of different parents for constructing the varieties.

Table 4. Similarity matrix showing the relationship among durum wheat genotypes based on RAPD data.

Varieties	Acsad 65	Bakrajo 1	Cemmitto	Creso Kurde	Ovanto	Creso Italy
Acsad 65	1,000					
Bakrajo 1	0,667	1,000				
Cemmitto	0,500	0,600	1,000			
Creso Kurde	0,632	0,783	0,571	1,000		
Ovanto	0,706	0,952	0,632	0,818	1,000	
Creso Italy	0,500	0,750	0,545	0,880	0,783	1,000

The introduction of molecular markers in plant breeding has presented a valuable tool for the characterization of genetic materials. Among them, the RAPD markers have been successfully used in wheat germplasm evaluation because of their many advantages. The suitability of the RAPD technique for genetic diversity studies and germplasm evaluations has been shown in many studies. The RAPD technique is quick (Colombo et al., 1998), cost effective (Fugang et al., 2003), and ab to perform analysis without need for prior sequencing of the genome (Huff *et* al., 1993). Although major bands from RAPD reactions are highly reproducible, minor bands can difficult to repeat due to random priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers (Tessier et al., 1999). Replication slippage is thought to occur more frequently than single nucleotide mutations and insertion\deletion events, which generated the polymorphisms detected by RAPD analysis (Powell et al., 1996). Various numbers of primers have been used in the study of different species of the genus *Triticum* that revealed various degrees of polymorphism. Joshi and Nguyen (1993) used 40 primers in studying wild, and cultivated wheat and revealed 88% polymorphism among all accessions. With 26 UBC primers, Sun et al. (Sun et al., 1998) detected 62.5% polymorphism among 46 genotypes of *T. aestivum* and *T. spelta*. Pujar et al. (1999) tested 81 Operon primers (kit A, F, J, V) and selected 21 primers that produced 3 to 13 polymorphic bands. A 78.2% polymorphism was detected among 64 genotypes of the species *Triticum.*

Figure 3. Dendrogram of durum wheat varieties showing genetic similarity, based on RAPD data by using UPGMA cluster analysis



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Genotoxic Effects of Catha edulis (Khat) Extract on Mice Bone Marrow Cells

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Abstract

Khat (*Catha edulis*) is a widespread habit that has a deep-rooted sociocultural tradition in the Horn of Africa and southwestern of Arabian Peninsula causing many social and economic problems. The genotoxic effects of methanolic extract of Khat (*Catha edulis*) leaves were investigated. Our results demonstrated a significant increase in sister chromatid exchanges SCEs in all treatments. This increase was more at higher concentrations than those occurred in lower concentrations. Moreover, Khat induced various types of chromosomal aberrations in mice bone marrow cells. These aberrations include: broken, sticky and ring chromosomes and disturbed metaphase and anaphase. The percentages of these aberrations increased with the increase of both concentration and the exposure time. Other types of aberrations were also noticed but in very low frequencies.

الملخص

يعتبر مضغ القات في المجتمعات الافريقية و جنوب غرب الجزيرة العربية من العادات المتجذره في هذه المناطق مسبباً العديد من المشاكل الاجتماعية و الاقتصادية، و يهدف هذا البحث الى دراسة السمية الوراثية لمستخلص نبات القات حيث تبين ان المستخلص يؤدي الى ارتفاع ذي دلالة احصائية في معدل تبادل الكروماتيدات الشقيقه وان هذه الزيادة تزداد بازدياد تركيز المستخلص، كما أن المستخلص أدى تلى ظهور أنواع مختلفه من أنواع الشذوذ الكروموسومي وتشمل: الكروموسومات الحلقية، الكروموسومات اللزجة، الكروموسومات المتكسره، الكروموسومات المضطربه، و نسبة هذه الانواع تزداد بازدياد كل من تركيز المستخلص والمدة الزمنية، كما ظهرت أنواع اخرى من الشذوذ ولكن بمعدلات متدنية.

Key words: Genotoxicity; Khat; Catha Edulis; Mice Bone Marrow; Chromosomal Aberrations

1. Introduction

Catha edulis (Khat) leaves are used by millions of people worldwide, mainly in Africa and the south west of Arabian peninsula causing many social and economic problems (Osborne, 1983). The alkaloid fraction of Khat is very efficiently extracted by chewing, and the major compounds are absorbed in the oral cavity (Toennes et al., 2003). Its stimulating effects as well as the psychological reaction induced among users have been reported by Kalix (1990). It was believed that the problem might be social rather than medical. But in some regions, a large proportion of population was spending great deal of the family income on Khat rather than on food, with the consequence that the consumers and their families suffered from malnutrition and weakness (Kabarity and Mallalah, 1980). Khat chewing during pregnancy may be one of the factors contributing to infant mortality in communities, where Khat is commonly chewed as well as Khat consumption affects the potency of male sexuality by affecting spermatogenesis and plasma testosterone concentration (Mwenda et al., 2003).

Toxicological evaluation of *Catha edulis* leaves has been reported by Al-Habori et al., (2002). Moreover, the toxicological potential of Khat has been reported by Carvalho (2003).The detrimental effects of the active principle Khat on man and animals have been described by Kalix and Khan (1984), and its mutagenic activity has been demonstrated by Hannan et al. (1985). Moreover, fresh leaves are chewed to produce an amphetamine like alkaloid , known as cathinone (AL-Ahdal et al.,1988). The active principle from *Catha edulis* (Khat) induced clastogenic effects in bone marrow of mice (Tariq et al., 1987).

Anti-gastric ulcer and anti-inflammatory activities of Khat have been also reported by Al-Meshal et al., (1983, 1985). The pharmacological activity of Khat has been described by several workers (Balint et al.1991; Kalix, 1991; Nencini and Ahmed,1989 and Widler et al., 1994). The biochemical activity of Khat has been described by Ahmed and El-Qirbi (1993) and its psychological effect

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has been reported by Mclaren (1987), Dhadphale and Omolo (1988).

Antimicrobial and cytotoxic activity of Khat extracts have been reported by Elhag et al. (1999). Moreover, it has been shown that cathinone isolated from *Catha edulis* (Khat) induced mitodepressive effect on the meristematic region of *Allium cepa* root tips (Al-Meshal, 1987).

Because of its adverse effects on human, Khat was classified by the World Health Organization in 1962 as a 'substance of abuse' (Kabarity and Mallalah, 1980; Giannini and Castellani, 1982). Indeed, a large number of medical problems have been reported in Khat chewers (Nencini et al., 1986; Granek et al., 1988; Eriksson et al., 1991; Widler et al., 1994) such as oral cancer (Soufi et al., 1991). Moreover, the incidence of head and neck squamous cell carcinoma seems to be relatively high, especially the oral squamous cell carcinoma, which may be considered as an important contributing factor (Nasr and Khatrl , 2000). Accordingly, investigation of harmful effects i.e. genotoxic effects of Khat should be done by sensitive and reliable method for detecting its genotoxicity.

It has also been reported that Khat induces cytotoxic effects in cells (Al-Ahdal et al. 1988; Al-Meshal et al. 1991; Al-Mamary et al. 2002; Dimba et al. 2003) in lymphoid tissue and in the liver and kidney of rabbits (Al-Meshal et al., 1991; Al-Mamary et al., 2002). Recently , the effect of Khat extract on three leukemia cell lines (HL-60, Jurkat and NB4 cells) was reported to be cytotoxic and induced a rapid cell death effect (Dimba et al., 2003). It also induced apoptosis through a mechanism involving activation of capase-1, capase-3 and capase-8 (Dimba et al. 2004).

The cytological effects of *Catha edulis* in somatic and male germ cells of mice have been demonstrated by Qureshi et al. (1988). They found that Khat significantly increased the frequency of micronucleated polychromatic erythrocytes; and induced bone marrow depression, and reduced the mitotic index of the somatic cells. Khat also induced significant-chromosomal aberration, namely; aneuploids, autosomal univalent, univalent of the sex chromosomes, and polyploids.

Khat consumption leads to formation of miconuclei in human buccal and bladder mucosa (Kassie, 2001). Relatively, little information regarding the genotoxic effects of Khat is available. The genotoxic potential of Khat has been carried out by Tariq et al. (1986) in Swiss albino mice. They studied the effect of Khat during the different stages of spermatogenic cycle and on the rate of pregnancy and post implantation losses. They found that Khat reduced the percent pregnant rates and increased the mean post-implantation losses in treated group. The increase was found to be statistically significant in postmeiotic stages.

There are numerous genotoxic bioassays, but each has its own specific attributes and limitations. Therefore, appropriate bioassays must be used in order to determine the genotoxic properties of Khat.

Numerical chromosome aberrations induced by khat extract in somatic cells of mice have been reported (Qureshi et al. 1988) while blood samples were analyzed for chromosomal aberrations assay by AL-Zubairi et al. (2008).

In view of the above, it is clear that understanding of Khat effect is of a particular interest. For this purpose, the present work is planned in order to investigate the capability of Khat in inducing genotoxic effects on mice genome. Sister chromatid exchange (SCE) and chromosome aberration test are selected and will be employed to achieve this purpose. It is obvious from the literature that genotoxic effects of *Catha edulis* (Khat) extracts in terms of their capacity to induce sister chromatid exchange (SCE) have not been investigated before.

2. Materials and Methods

The dried material of *Catha edulis* (Khat) leaves was grounded and powdered using an electric grinder. The powdered material was then extracted using a soxhlet extraction apparatus. The air dried powder of *Catha edulis* was extracted continuously for 24h in a soxhlet extraction apparatus with a range of solvents, with n-hexane (to separate lipids and terpenoids); with ethyl acetate (for separation of more polar compounds), and then using methanol (for separation of the polar compounds) as in the extraction procedures according to Ayoub et al. (1989).

A series of concentrations of the methanol extract were prepared 10,25,50, and 100 mg /kg, and their genotoxic effects on bone marrow cells of Swiss male white mice (*Mus musculus*, 2n= 40) were tested for different periods of time after a single intraperitonial injection.

All dosing solutions we administered were at a volume of 0.1 ml/kg body weight (b.w.). Animals in the negative control groups received an equivalent volume of normal saline. Mitomycin c (MMC) was used as positive controls. Metaphase bone marrow cells were prepared for mitotic investigation by the classical method. The preparations were stained with Giemsa solution, pH 6.8, as described by Allen et al. (1978). Slides were also scored for chromosome aberration. Evaluation of genotoxic effects of Catha edulis (Khat) extracts, in terms of their capacity to induce various types of chromosome aberrations, was studied.

2.1. Sister Chromatid Exchange (SCE):

For each dose, four animals weighing 20-25 g were used .At least 400 somatic bone marrow cells of second metaphase were analyzed. The bromodeoxyuridine tablets were prepared and implanted subcutaneously to Khat treated animals with 10, 20, and 40 mg/kg body weight. Bone-marrow harvest, and slide preparations were performed as described by Allen et al. (1978).

The method of Goto et al. (1978) was used in order to obtain differential staining of sister chromatids. SCE frequencies were counted from the microscope images of the second division cells. An interstitial exchange segment was counted as 2 SCEs. Mitomycin C (MMC) was used as a positive control because of its ability to induce SCEs while dimethylsulfoxide (DMSO) was used as negative control. Students t-test was used to compare the level of significance of the results for the Khat-treated groups and the untreated control as well as the various treated groups.

3. Results

Catha edulis (Khat) extract induced a significant increase in the frequency of SCEs (P<0.001) in bone marrow cells as compared with untreated controls at all three doses (10, 20 and 40 mg/kg) (Table.1). However, no significant difference could be found in the frequency of SCEs among treated groups. Moreover, the potency of *Catha edulis* (Khat) on induction of SCEs was significantly lower than that caused by the positive control (MMC) by almost the 50% in all treatments (Table.1).

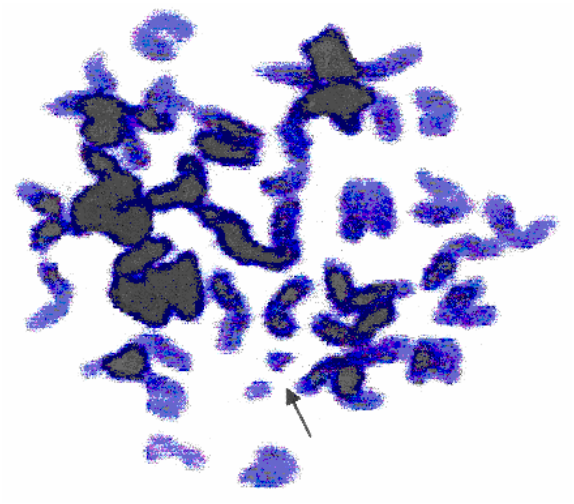

Figure 1. broken chromosomes (arrow) after treatment with 100 mg/kg b.w. aqueous extract from Khat (*Catha edulis)* for 48 hrs. Bar represents 5 µm in length.

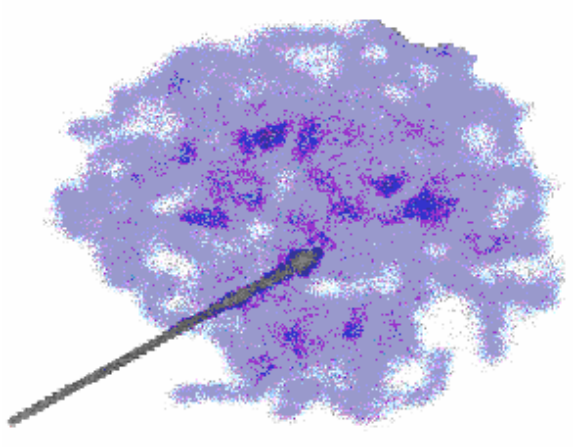

Figure 2. sticky chromosomes (arrow) after treatment with 100 mg/kg b.w. aqueous extract from Khat (*Catha edulis)* for 48 hrs. Bar represents 5 µm inlength.

The ability of Khat extract to induce the formation of chromosomal aberrations was assessed using mouse bone marrow cells. Scanning of the chromosomal aberration concentrated on the followings; broken chromosomes (Figure1), sticky chromosomes (Figure2), ring chromosomes (Figure3), and chromatid disturbances (Figure4). Other types of chromosomal aberrations such as fragments, micro, and macronuclei and bridges were also noticed but in very low frequencies.

Statistical analysis of the data (t-test, P< 0.001) revealed that there is a significant difference between Khat treated groups at different concentrations and exposure times and the control group (Table 2). Moreover, in the three exposure times, the percentages of chromosomal aberrations in the treated groups were significantly higher than those of the control. These percentages increased with increased concentrations as well as with increased exposure times (see Table 2).

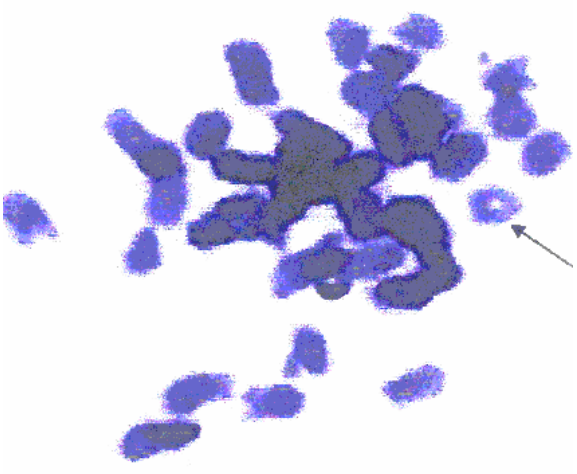

Figure 3. ring chromosomes (arrow) after treatment with 100 mg/kg b.w. aqueous extract from Khat (*Catha edulis)* for 48 hrs. Bar represents 5 µm in length.

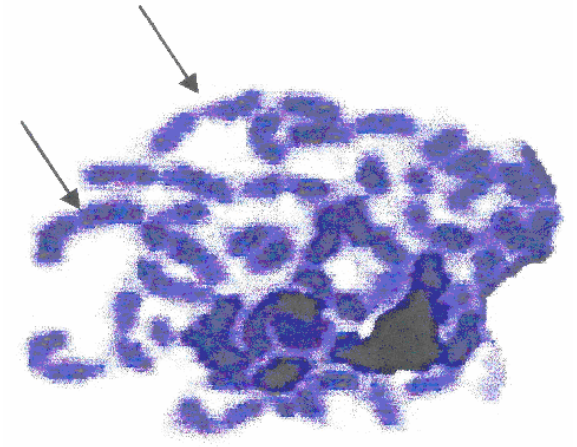

Figure 4. Disturbed metaphase and anaphase (arrows) after treatment with 100 mg/kg b.w. aqueous extract from Khat (*Catha edulis)* for 48 hrs. Bar represents 5 µm in length.

4. Discussion

Catha edulis (Khat) leaves are used by millions of people as a social habit, and there is little information about its biological activity (Carvalho, 2003). The aim of this study was to investigate the genotoxic effects of Khat extract as measured by chromosomal aberrations of the following parameters: sister chromatid exchange (SCE) and chromosomal aberration.

Table 1. Effects of Khat extract on the frequency of SCEs in the bone marrow cells of mice.

Dose (mg/kg) b.w	Cells examined	SCE/ CELL	Mean ±S.D
	100	5.27	
10	100	5.15	
	100	5.33	
	100	5.56	5.33 ± 0.27*
20	100	5.51	
	100	5.67	
	100	5.50	
	100	5.22	5.48 ± 0. 18*
40	100	5.81	
	100	5.51	
	100	5.80	
	100	5.94	5.77± 0.26*
MMC 2.0	100	10.36	
	100	10.51	
	100	10.55	
	100	10.60	10.55± 0.14
DMSO	100	3.15	
	100	3.47	
	100	3.77	
	100	3.85	3.56± 0.24

*Significantly different from control ($p < 0.001$)

Sister chromatid exchange (SCE) test is widely used in genetic toxicology, and therefore the induced SCE is of great importance (Bruckmann et al. 1999). Moreover, it is considered a sensitive indicator of genetic toxicity (Khalil, 1996).

The present study of Khat extract effect on bone marrow cells of mice has revealed that Khat extract causes significantly increased frequencies of SCEs ($P < 0.001$) in cells treated as compared with the controls at all three doses (10, 20, and 40 mg/kg) (Table 1). However, no significant difference could be found among treated groups. Similar increase in the frequency of SCEs induced by alkaloids has been reported by Das et al. (2004) on their study using Sanguinarine (SG), a benzophenanthridine alkaloid. They found that (SG) increased sister chromatid exchange frequencies. It is also reported that the major alkaloid of betel nut, arecoline (ARC) induced a high frequency of SCEs after oral administration (OA) and intraperitoneal injection (IP) in mouse bone marrow cells (Chatterjee and Deb, 1999). Moreover, Boldine which is an alkaloid present in *Peumus boldus* (populary called "boldo- do- chile" in Brazil) which has healing properties; and is used for the treatment of gastrointestinal disorders induced SCEs when tested *in vitro* on human peripheral blood lymphocytes (Tavares and Takahashi, 1994).

Khat consumption caused genotoxic effect in humans (Kassie et al. 2001). It also caused cytotoxic effects in bone marrow cells of mice treated with 125, 250, and 500 mg/kg (Qureshi et al. 1988).

SCEs arise from reciprocal exchange of DNA at apparently identical loci of the sister chromatids of a duplicated chromosome in response to a damaged DNA template. The frequency of SCEs in eukaryotic cells is increased by exposure to genotoxic agents, which induce DNA damage that is capable of interfering with DNA replication (Tucker et al. 1993). The significant increase in the frequency of SCEs induced by Khat extract may further indicate the potential interaction with cellular DNA. So, the present data clearly indicates that (Khat) possesses the potential, at least to a limited extent, to cause alterations in cellular DNA in mice cells *in vivo*.

In the current study, the percentage of chromosomal aberrations in cells after 4 h exposure with the control (DMSO) was 0.24 (Table 2). This percentage was elevated to 0.45 and 0.53 when exposure time was increased to 24 h and 48 h respectively. These results are in accordance with those reported by Tavares and Takahashi (1994) on studying genotoxic potential of the alkaloid boldine in mammalian cell systems *in vitro* and *in vivo* using blood samples from healthy people.

Significant differences in the percentage of chromosomal aberrations relative to the control were also observed when alkaloids were applied in almost all treatments. After 6 h exposure, the percentage was 0.74 at the lowest concentration (10 mg/kg b.w.), this was elevated to 3.10 at the highest concentration (100 mg/kg b.w.). This trend of elevation was also observed at the other two exposure times 24 h and 48 h (Table 2).

Referring to the same table, it is clear that the percentage of chromosomal aberrations increases with increased concentration as well as increased exposure time. Similar results were obtained by AL-Zubairi et al. (2008) on studying the genotoxic effect of Khat in rats. However, we observed concentration dependent chromosome aberration frequencies. These results are also in agreement with those reported by Ribeiro et al. (1993) on studying the effect of extracts obtained from *Crotalaria retusa* on mouse bone marrow cells.

Therefore, we can suggest that *Catha edulis* (Khat) contains some mutagenic and potential carcinogenic agents. Culvenor et al. (1962) presented evidence that the effects of alkaloids on cell nuclei are due primarily to their ability to act in the cell as alkylating agents.

Linearly along with increasing concentrations of akkaloids as well as *Catha edulis* (Khat) extract induced the formation of various types of chromosomal aberrations. The most common abnormality is the broken chromosome as shown in Figure1. Chromosomal breaks result from the action on the DNA synthesis (Evans, 1969).

Broken chromosome percentages are increased linearly along with concentrations used; and are also increased through different periods of time in all cases.

Table 2. Chromosomal aberrations *in vivo* bone marrow cells of mice treated with different concentrations of Khat extract for 6, 24 and 48h.

Exposure (h)	Dose (mg/kg) b.w	Cells examined	Aberration types				
			Disturbed metaphase and anaphase	Ring chromosomes	Broken chromosomes	Sticky chromosomes	%*
6	DMSO[a]	4120	-	4	6	-	0.24
	10	4200	-	15	16	-	0.74
	25	4000	1	26	30	15	1.80
	50	4050	2	26	40	30	2.42
	100	4100	6	25	45	51	3.10
24	DMSO[a]	4000	2	7	8	1	0.45
	10	4266	4	13	22	2	0.96
	25	4520	8	26	45	18	2.15
	50	4030	9	35	55	26	3.10
	100	4150	12	42	70	48	4.18
48	DMSO[a]	4000	1	8	9	3	0.53
	10	4008	6	16	23	7	1.30
	25	4222	9	28	48	18	2.44
	50	4175	12	42	60	38	3.64
	100	4250	12	48	71	55	4.38

*%: Total No. of chromosomal aberrations / Total No. of cells examined. Control= 10 µl

This suggests that alkaloidal fraction may contain alkylating compounds (S-dependent agents) that produce aberrations *via* misreplication (DNA damage happened when a DNA molecule with lesions undergoes DNA replication) (Palitti, 1998). This suggestion is supported by Peter et al. (2002), who found a variety of alkaloids in *Astraceae* family plants that produced genotoxicity.

Sticky chromosomes occurred at a high percentage with extract treatments (Figure 2). The percentage of this type of aberration is also increased linearly with the concentration and through time of exposure.

Stickiness has been attributed to an action on the proteins of chromosomes (Badr, 1982), and may be due to the increase in viscosity of the cytoplasm (Abderrhman, 1998). This finding is in line of Al-Meshal (1987) when he tested the effect of cathinone, from *Catha edulis* (Khat) on *Allium cepa* root tips. Cathinone produced significant sticky chromosomes.

Ring chromosomes (Figure 3) induced in a considerable percentage, and is increased linearly to reach a highest percentage with the highest concentration of alkaloids at 48 h exposure time (Table 2). These results are in line with Abderrahman(1998) on studying the effect of *Peganum harmala* extract on Maize root tips. Ring chromosomes results from stickiness (Evans, 1969) and double strand breakage (lesions), and also id due to exchange type of interaction, which takes place between the two lesions after formation of a looped structure (Bryant, 1998).

Disturbed metaphase and anaphase (Figure4) were also noted in almost all treatments. The formation of disturbed metaphase and anaphase might be due to a disturbance in the mechanism of chromosome movement (Abo-El-Khier and Abo-ElKhier, 1992). Other types of chromosomal aberrations such as fragments, micro, and macronuclei bridges are also noticed but in very low frequencies. Khat extracts treatment cause an increase in aberrant metaphases. Chromatid gaps were shown to be the most frequent type of aberration followed by chromatid breaks. Similar abnormalities were reported by Geri et al. (2002) while acentric fragments were observed to be less frequent when they evaluate the genotoxic effects of crude extract of Khat leaves after 2000 mg/kg treatment.(AL-Zubairi et al., 2008)

Thus, various types of chromosome aberrations were induced by *Catha edulis* (Khat). The percentage of these abnormalities is increased with the increases of both concentration and exposure time in all treatments. Similar increase in the total percentage of total abnormalities in *Vicia faba* and *Allium cepa* after treatment with Vinca alkaloids was reported by Abed EL Tawab (1983). Moreover, the results obtained from this study are in line with Abu El Kheir and Abu El Kheir (1992) on studying the effect of harmole and harmine alkaloids extracted from *Peganum harmala* on mitosis of *Allium cepa.*

Mitodepressive effect of Khat on bone marrow of mice has been reported by Omari et al. (1996). Thus, the decrease in mitotic index in higher concentrations might be due to the action of alkaloids on the onset of mitosis which differ from the action of colchicine in its action.

In conclusion, the present study indicates that *Catha edulis* (Khat) extract probably has some interactions with DNA metabolism in mice, resulting in SCEs and suggesting potential mutagenic effects. Moreover, the

present study suggests that Khat is a potent genotoxic agent. Thus, additional studies under various conditions would be helpful in placing the magnitude of genotoxic and cytotoxic responses to Khat extracts in proper perspective.

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Impact of Summer Thermal Stratification on Depth Profile of Phytoplankton Productivity, Biomass, Density and Photosynthetic Capacity in Lake Nasser (Egypt)

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Abstract

Lake Nasser is a headwater of Nile River in Egyptian territory. In wintertime, there is a complete upwelling in the water body whereas it is clearly thermally stratified in summer. Depth profile of summer stratification at the upper 15 m was investigated in four different sites. Temperature amplitude reached about 10 °C whereas oxygen concentration was about 7.491 mgO_2 l^{-1}. Epilimnion layer was extended to 10 m depth whereas metalimnion underwnt from about 10 to 15 m and hypolimnion was initiated down. Bands of stratification were affected with the inflow of River Nile at the south. Peak maximum of chlorophyll *a*, phaeophytin, and standing crop was recorded at 10 m. Net primary productivity was irregular along the depth profile. Superficial water had climax photosynthetic capacity and declined downward. Integrated column productivity was correlated with that equivalent concentration of chlorophyll *a*. Khore Korosko exhibited the highest integrated productivity rate (315.9 mgC m^{-2} h^{-1}) and photosynthetic capacity (24.4 mgC $mgChl^{-1}$ h^{-1}) among the studied sites. Layering width of water stratification and its temporal occurrence were affected with currant and physics of flooded water. So epilimnion is usually shallow in the north section while it becomes deeper toward the inflow of Nile floodwater at the south.

الملخص

بحيرة ناصر هي خزان لمياه نهر النيل في مصر.عامة تمتزج مياه البحيرة كليا خلال موسم الشتاء، بينما تتسم بظاهرة التدرج الحراري خلال الصيف. تم دراسة القطاع الرأسي لمياه البحيرة بعمق 15 متر خلال هذه الظاهرة من خلال أربعة مواقع والتي أسفرت عن: تراوح تذبذب درجة حرارة المياه فى حدود 10 درجات مئوية، في حين كان نطاق التدرج فى الأوكسجين الذائب فى حدود 7.491 mgO_2 l^{-1}. امتدت الطبقة السطحية إلى عمق حوالي 10 أمتار، في حين أن الطبقة الوسطي تراوحت ما بين 10 إلي 15 متر، ثم تندرج بعد ذلك العمق الطبقة السفلى (الأكثر كثافة). يتأثر سمك هذه الطبقات بمعدل فيضان نهلا النيل. سجلت أعلى تركيزات لصبغ الكلوروفيل و الفيوفيتين وكذلك كثافة العوالق النباتية عند عمق 10 متر.بينما كانت قيم حصيلة الإنتاجية الأولية غير منتظم في مختلف الأعماق. لكن سجلت الطبقة السطحية من مياه البحيرة أعلى معدل لعملية البناء الضوئي ثم تضاءلت في الطبقات السفلى. وقد تناسب التقدير التراكمي لإنتاجية وحدة المساحات للأعماق المختلفة فى القطاع الرأسي للمياه مع تركيزات الكلورفيل أ، و التي سجلت أعلى قيمة لها (315.9 mgC m^{-2} h^{-1}) فى مياه خور كوروسكو، وكذلك أعلى معدل للبناء الضوئي (24.4 mgC $mgChl^{-1}$ h^{-1}) مقارنة بالمواقع الأخرى.وقد تبين من المسح الأفقي و الرأسي لهذه الظاهرة أن سمك هذه الطبقات و توزيعاته الجغرافية تتوقف على طبيعة مياه الفيضان و سرعة معدلاتها. ولذلك يلاحظ ضآلة عمق الطبقة السطحية لمياه البحيرة في الشمال، بينما يزداد عمقا كلما اتجهنا إلى الجنوب باتجاه مياه فيضان نهر النيل.

Key wards: Lake Nasser; Stratification; Phytoplankton Biomass; Photosynthetic Capacity

1. Introduction

After construction of the High Dam, in 1968 for flood protection at Aswan city (Egypt), the High Dam Lake was formed as a newly created headwater of Nile River. It is one of the largest man-mad lakes in the world and among four largest African man-made reservoirs. A reservoir that has been used for many purposes including water supply, hydro-electric generation, and fish production. Even the smallest of these reservoirs exhibited a measurable thermal stratification during summer. Surface water quality standards of dissolved oxygen concentration for reservoirs only apply to the surface mixed layer. Lack of oxygen in the deeper water limits aquatic life uses and may produce other undesirable water quality conditions.

Primary productivity, a division of biological productivity dealing with transformation of solar energy to

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the potential energy of organic protoplasm, is the best studied aspect of biological productivity (Saunders et al., 1962). Importance of primary productivity measurements in aquatic environment is well established. It would be obvious that fish production in natural and tended waters should be related to primary production of water bodies concerned. In African lakes, it has been found that fish yield is well correlated with corresponding primary production (Melack, 1976).

Chemical and physical measurements tend to measure only the cause of change in water quality while biological tests deal primarily with effects of the change. Phytoplankton composition and density can be considered as an indicator of trophic status of the lakes. Seasonal changes in environmental condition greatly affect fluctuations in phytoplankton flora of the water. While regional variations of phytoplankton reflect its response to varying environmental conditions and this is affected by advection, turbulence, and grazing (Chang and Bradford, 1985). Other wise, Chlorophyll a (Chl a) is one of the most effective variables on eutrophication of aquatic ecosystem. Its measurement is much easier, which serves as an integrative measure of phytoplankton biomass and of photosynthetic potential. And thus a number of trophic-state prediction models which are based on phosphorus-chlorophyll relations (Horne and Goldman, 1994).

Temperature measurements occupy a central position in limnology, as its changes may affect many physiological processes, the density of water, and fundamental stratification of a water body. Water temperature has extremely important ecological consequences. It has influence on water chemistry. It is a regulator of gases and minerals solubility in water body. Solubility of important gases like oxygen and carbon dioxide increases as temperature decreases. Inversely, solubility of most minerals increases with increasing temperature. It exerts a major influence on aquatic organisms with respect to selection/occurrence and level of activity of the organisms. All aquatic organisms prefer temperature in which they can survive and reproduce optimally (Lund and Talling, 1957).

While thermal stratification is a process that occurs in natural lakes, a case which is made for managing undesirable aspects of this process Water thermal stratification, or layering, have been occurred in some Egyptian lakes, particularly in the warm months. It was recorded in Lake Nasser (Abd El-Monem, 1995), Wadi El-Rayan (Taha and Abd El-Monem, 1999) and Abo-Zabal Aquatic Depressions (Abd El-Monem et al. 2006). Lake stratification depends on a number of factors like shape and depth of the lake, the amount of wind, and orientation of the lake (Entz and Latif, 1974). It is often paralleled by stratification of other water quality measurements (chemical and biological).

Primary productivity was discussed using 14C technique along the main channel and was discussed with phytoplankton biomass, density, and some physico-chemical variables during 1993 (Abd El-Monem, 1995). He reported that water column was well thermally stratified during hot months with maximum amplitude during summer. In addition, he stated that water temperature was the most important factor that regulates ecology of aquatic ecosystem in the lake, especially phytoplankton density and activity in the water column during thermal stratification.

Primary productivity of the Egyptian lakes, which has a wide range of trophic status, has been inadequately covered. It was highly temperature dependence (Abd El-Monem, 2001). The present study on Lake Nasser was designed to investigate some ecological variables that control water fertility and fish production, especially in Khores. The temperature profile depicts this layering for a hypothetical summer profile and its role on water variables. To estimate temperature amplitude in the water column, and thermocline impact on depth profile of dissolved oxygen, phytoplankton productivity, biomass, density and photosynthetic capacity. Integration for the water column production, per surface area, at the different khors and evaluate which is the most productive one.

2. Material and Methods

2.1. Lake Morphometry and Site Discretion

To study the primary productivity of the aquatic ecosystem in Lake Nasser, topography and ecology of the lake must be introduced to indicate its ecological status. The High Dam Lake is a newly created headwater of Nile River formed after construction of the High Dam (1968) at Aswan city (Egypt). It is one of the largest man-mad lakes in the world and among four largest African man-made reservoirs (extended about 496 km and occupied about 6275 km^2). Lake Nasser is the largest part of the High Dam Lake in Egyptian territory (292 km long) while the other is Lake Nobia in Sudan (204 km long). It lies in subtropical arid region and extends between latitude 22° 00′ – 23° 58′ N and longitude 31° 19′ – 33° 19′ E. It has an area about 5248 km^2, mean depth about 21.5-25.5 m and width about 8.9-18.0 km, at 160 and 180 m above the main sea level, respectively. The Lake morphometry is shown in Table (1). According to its topography, it has great variations in its ecological nature. It has many employments locally called Khors as shown in Figure (1). There are 85 important Khors (48 on the eastern side and 37 on the western). Those Khors covered about 4900 km^2, constituting about 79 % of the total lake surface area outside the main valley and contained only 86.4 km^3 water, forming about 55% of the total lake volume. Kalabsha, Tushka, and Allaqi are the widest Khors with slope gently while El-Sabakha and Korosko are steep with relatively narrow (Bishai et al., 2000).

Lake Nasser is one of the main sources of fish yield in Egypt. Its amount peaked to 34,206 ton of fresh fishes cached during 1981 as recorded in Bishai et al. (2000). Ecological natures of water body in Khors are different than those characterize the main channel. Khors are highly productive, and most of fish landing and catches from it. In the main channel, water column is deep, with a maximum depth (about 130 m) in the north at the High Dam; and decreased gradually downward while it was shallow in khors.

The present study was designed to represent water body and depth profile in the main channel and Khors during summer thermocline during July (2005). Samples were collected from the upper 15 m depth (the approximate mean depth of the photic zone) at different depths: 0, 3, 5,

10 and 15m from four sites. One represented the main channel and located in El-Madiq. The other three represented the khors (kalabsha, Korosko and Tushka) and located in the deepest position. The sampling sites are shown in Figure (1).

Results were expressed at the given depths per unit volume (m^3); and were integrated to calculate it per unit area (m^2) for that layer. The main morphometric variables of the selected locations are illustrated in Table (1) as reported in Bishai et al. (2000).

Site name	Distance from High Dam (km)	Length (km) at 160 and 180 m		Surface area (km^2) at 160 and 180 m		Perimeter (km) at 160 and 180 m		Maximum depth (m)
Kalabsha	46	22.0	47.2	54.0	620.0	85	517	40
El-Madiq	127	Main channel						54
Korosko	177	10.7	22.56	23.4	83.6	34	253	30
Tushka	245	24.1	33.35	49.1	366.8	89	127	20

Table 1. General morphometry of the studied sites in Lake Nasser at 160 and 180 m (above sea level) water levels. (cf. Bishai *et al*, 2000).

2.2. Field Measurements

Water depths were measured using Portable Echosounder (Lowrance x25). Water temperature and pH were measured in situ using Environmental Monitoring System (YSI-3800). A black and white standard Secchi-disc (25cm diameter) was used in measuring water transparency.

2.3. Collection and Preparation of Samples

Water samples were collected with water sampler (Ruttner, 1.5 liter) equipped with mercury thermometer. BOD bottles were filled carefully with the collected water samples, dissolved oxygen (DO) was fixed, and two others were prepared for primary productivity measurements as will be discussed below. For pigments analyses, a definite volume of water sample was filtered on glass microfiber filter (GF/F), using filtration unit (Sartorius). The filters with the remnants were foiled and preserved (refrigerated) for pigments analysis in the laboratory. One litter of sample was fixed in 4% neutral formalin and Lugol's iodine solution for quantitative analysis of phytoplankton, as described by Margalef (1974).

2.4. Methods of Analysis

DO in the lake was measured using Winkler method. It was analyzed and calculated as maintained by Thompson and Robinson (1939). Oxygen content is used as the initial concentration in calculating productivity.

Net primary productivity was measured as amount of DO increased in specific water volume during a period of time. Light and dark bottles technique was applied as described by Vollenweider (1969). Two sealed white bottles were immersed in the water body and incubated for 4 hours (mid day hours) at the same level of the water where sample is taken. After the incubation period, DO of the incubated bottles was fixed and measured. Net primary productivity was calculated as the difference in DO between light and initial bottles. Rate of oxygen released can be converted to rate of carbon uptake by calculation from the summary equation of photosynthesis

$$6CO2 + 6H2O \rightarrow 6O2 + (COH2)6 \quad (1)$$

So the values in $mgO_2\ l^{-1}$ are converted to $mgC\ l^{-1}$ by multiplying by the factor 0.735, the ratio of the weight of carbon to oxygen (Moss, 1980).

Phytoplankton biomass was represented as Chl *a* content. The preserved samples of the remnants residue on filters (G/F) were extracted in 90% acetone overnight at 4 ºC as described by Parsons et al. (1984). To estimate Chl *a* concentration, the extract was centrifuged and measured with spectrophotometer (Double beam, Kontron 930, UV&Vis) at the different wave lengths required for applying trichromatic equation as reported in SCOR/UNESCO (1991). The extract was acidified with dilute HCl and re-measured to calculate phaeophytin.

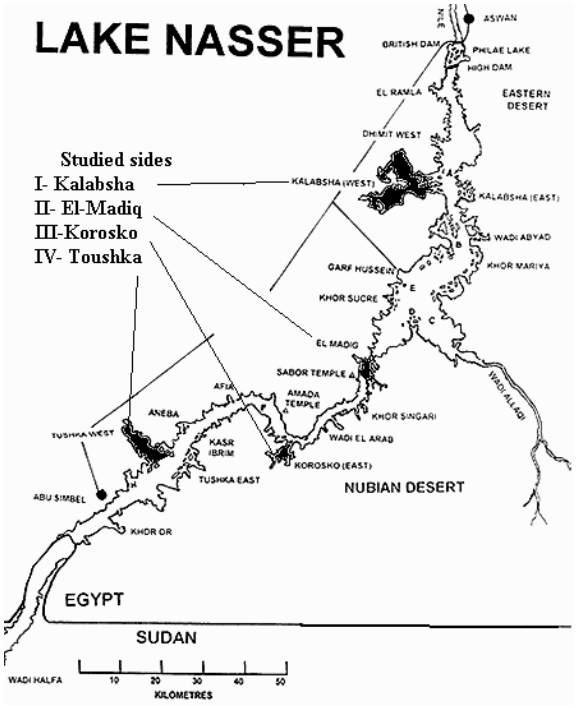

Figure 1. Lake Nasser map with a remarkable studied sites for summer stratification.

For estimating phytoplankton density, preserved water samples were allowed to settle in glass cylinder for 5 days with faint tea color Lugol's Iodine solution. To concentrate the samples, 90% of the supernatant fluid was slowly siphoned off with narrow plastic tube covered with 10µ plankton net. Adjusted volume (10%) was transferred to a plastic vial for microscopic examination. Drop method was applied for phytoplankton counting (APHA, 1992). Inverted microscope (ZEISS 1M4738) with magnification power 40 and 100x was used.

3. Results

Table 2. Temperature and dissolved oxygen amplitudes, and integrated values of some biological variables for water columns at the studying sites in Lake Nasser during summer stratification.

Site	Amplitude		Integrated value for depths per unit area (m^2)				
	Temperature (°C)	Dissolved oxygen ($mgO_2\ l^{-1}$)	Chlorophyll *a* ($mg\ m^{-2}$)	Phaeophytin ($mg\ m^{-2}$)	Primary productivity ($mgC\ m^{-2}\ h^{-1}$)	Assimilation rate ($mgC\ mgChl^{-1}\ h^{-1}$)	Standing crop ($x10^6$ unit m^{-2})
Kalabsha	8.2	7.491	238.9	677.0	257.1	22.0	1713.7
El-Madyiq	10.0	6.744	206.2	535.7	238.0	23.8	2683.4
Korosko	9.9	6.878	247.0	667.9	315.9	24.4	2886.0
Tushka	6.4	5.278	231.1	571.5	253.4	18.5	2568.6

Secchi depth readings at the selected sites in Lake Nasser were taken the northern side of the lake water was more transparent than in the south. The highest Secch depth reading in the water column was about 3.15 m at Kalabsha. While it was about 76.19 % in the southern site reaching to about 2.4 m in Toshka. Trend of water visibility in the lake was decreased southward as shown in figure (2), which represents Secch depth readings in water of the different sites.

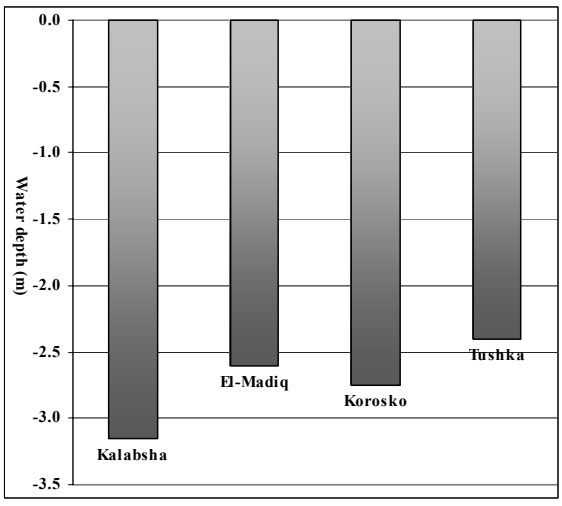

Figure 2. Water visibility (Secchi-depth readings) at the studied sites in Lake Nasser.

In contrast, water temperature of the lake was slightly increased southward. Local distribution of water temperature revealed that water was warmer upstream than in downstream. The highest water temperature was recorded at uppermost layer in Toshka of 31.8 °C. Whereas the minimum water temperature was 26.7 °C; and was recorded at the deepest studied layer (15 m depth) in El-Madiq. Depth profiles of water temperatures during summer are shown in Figure (3). It shows that water column was thermally stratified. Its amplitude, a difference between surface and bottom water temperatures, had the widest range of about 10.0 °C at El-Madiq when water temperature decreased gradually downward from 30.4 to 20.4 °C. While, amplitude was limited to 6.4 °C at Toshka when water temperature declined from 31.6 to 25.2 °C (see Table 2). It was indicated that water in the main channel, as represented by El-Madiq, has wide rang of temperature difference compared with that recorded in the Khors. Amplitude of water temperature in Khors decreased to about 64 % from that found in the main channel.

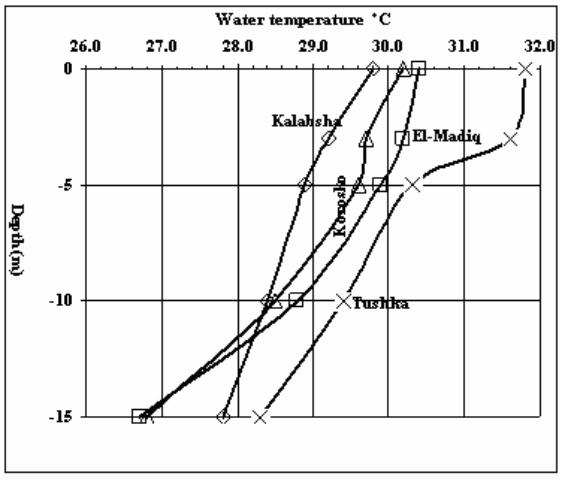

Figure 3. Depth profile of water temperature (°C) during summer stratification.

Oxygen contents of the studied water layers in Lake Nasser indicate that water column was oxycline, and epilimnion water was well oxygenated during summer. The highest values of DO were recorded in the subsurface layer at 5 m depth for most sites. Depth profiles of DO concentrations in water of the investigated sites, as represented in Figure (4), show that maximum concentration was 8.94 $mgO_2\ l^{-1}$ at 5 m depth in Kalabsha. Oxygen depletion was developed downward until it was deoxygenated in the hypolimnion layer with a concentration of 0.81 $mgO_2\ l^{-1}$ at 15 m depth in Korsko. Local and vertical distributions of DO in the water column with slight variations in oxygen contents through the upper 10 m depth were recorded whereas remarkable depressions were recorded downward in all sites. Oxygen depression in water column was maximized in Korsko getting to about 10.5 % from the one measured at 5 m depth. The differences were in oxygen contents between the maximum and minimum values in each water column, which can be defined as oxycline amplitude varied among the studied sites as illustrated in Table (2). The widest range of oxycline amplitude was about 7.491 $mgO_2\ l^{-1}$ at Kalabsha, whereas it was limited to about 5.28 $mgO_2\ l^{-1}$ at Toshka.

Spatial distribution of phytoplankton biomass, as represented by Chl a, is affected by the other environmental variables. Variations of Chl *a* in the similar depths were clear among investigated sites while its vertical distribution showed remarkable difference between the depths in the water column as illustrated in

Figure 5. Generally, surface water contained the lowest concentration of Chl *a*, compared with other investigated depths whereas the highest value was established at 10 m depths in all sites. Phytoplankton biomass in the lake water ranged from the least Chl *a* value of 4.67 mg m^{-3} at the surface water in El-Madiq to about 5.7 times at 10 m depth in Korsko, recording its highest absolute value that reached 27.01 mg m^{-3} (see Figure 5).

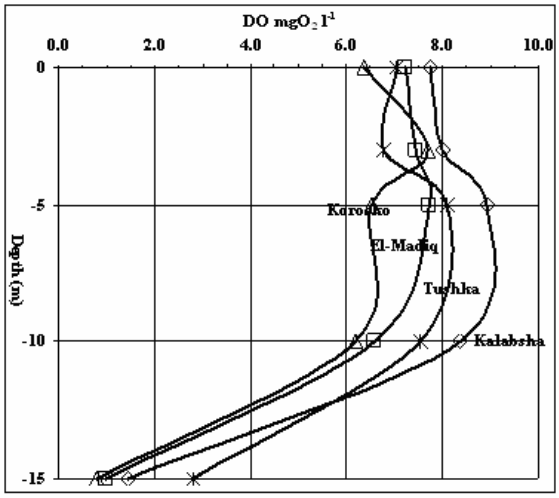

Figure 4 . Depth profile of dissolved oxygen (mgO_2 l^{-1}) during summer stratification.

Geographical distribution of phaeophytin pigment in the water columns, as represented in Figure (6), showed that It has the same trend of Chl *a* distribution; and is closely associated with its corresponding value in the studied layers. Phaeophytin concentrations were fluctuated from the minimum value of 12.334 mg m^{-3} to the maximum of 75.283 mg m^{-3} at its equivalent points of Chl *a*, respectively.

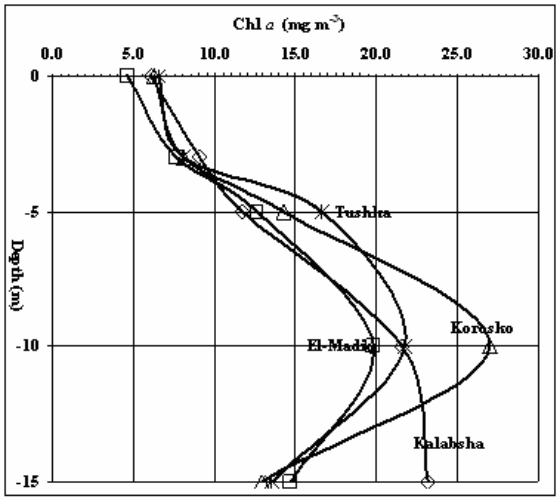

Figure 5. Depth profile of Chlorophyll *a* (mg Chl *a* m^{-3}).

Integrated concentrations of Chl *a* for the studied layers were fluctuateing from 206.2 to 247.0 mg m^{-2} at El-Madiq and Korsko, and Phaeophytin from 535.7 to 677.0 at El-Madiq and Kalabsha, respectively (Table 2).

Notable irregular variations in net primary productivity were observed along water column of the different sits. It was increased in the upper water layers (3 and 5 m) at two sites (Kalabsha and El-Madiq) and decreased downward. Whereas, a regular decrease in its value was recorded in the other two sites. Net primary productivity values fluctuated from the minimum of 10.6 mgC m^{-3} h^{-1}, at 15 m depth in El-Madiq, while the maximum was 24.8 mgC m^{-3} h^{-1}, at 3 m in Kalabsha (see Figure 7). Integrated values of net primary productivity for those depths at the studied sites were minimized to 238.0 mgC m^{-3} h^{-1} at El-Madiq and maximized to 315.9 mgC m^{-3} h^{-1} at Korsko as shown in Table (2).

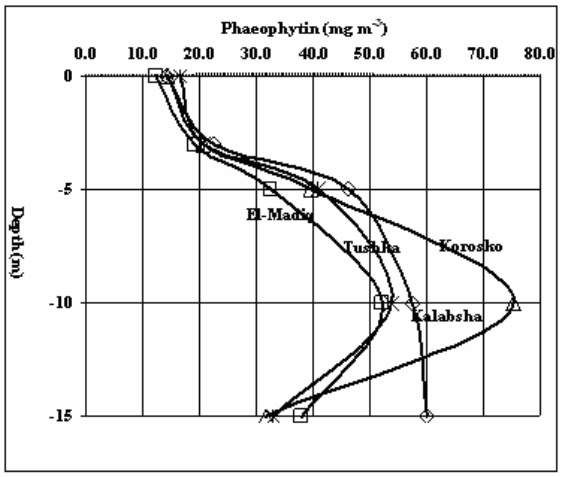

Figure 6. Depth profile of Phaeophytin (mg m^{-3}).

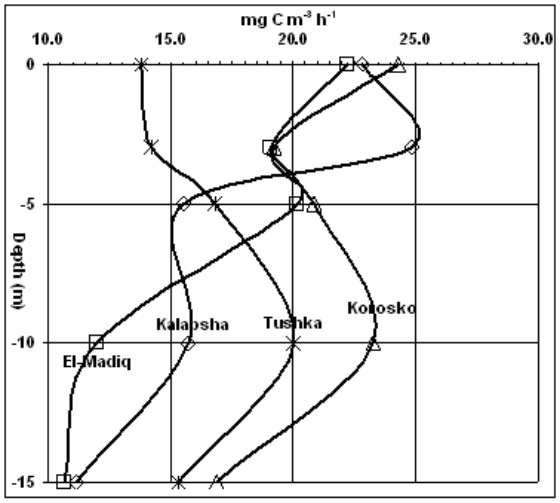

Figure 7. Depth profile of net primary productivity (mg C m^{-3} h^{-1}).

Photosynthetic capacity (assimilation rate) of the given sites in Lake Nasser is illustrated in Figure (8). In the studied sites, vertical and horizontal distributions of the assimilation rate values showed greet variations. Vertically, the highest assimilation rate was recorded at the upper water layer in all sites. Horizontally, limits of the assimilation rate varied from site to another. In general, assimilation rate in the studied sites varied from the maximum value of 4.753 mgC $mgChl^{-1}$ h^{-1}, which was recorded in El-Madiq to the lowest value of 0.479 mgC $mgChl^{-1}.h^{-1}$ recorded at 15 m depth at Kalabsha. Integration for its depth profile (Table 2) showed that, Korsko had the highest assimilation rate (24.4 mgC $mgChl^{-1}$ h^{-1}), and the lowest (18.5 mgC $mgChl^{-1}$ h^{-1}) was at Toshka.

Distribution of phytoplankton standing crop along water column (Figure 9) indicated that it was condensed at a depth of 10 m in most sites. Its highest density was about 278.8 x 10^6 unit m^{-3} which was observed at 10 m depth in Toshka. whereas it declined to the minimal density of about 28.7 x 10^6 unit m^{-3} at the deep water layer in Kalabsha. Integration of phytoplankton density in the water depth profile, at different locations, indicated that water column in Toshka contained the maximum density of phytoplankton, which was about 2886.0 x 10^6 unit m^{-2}, compared to with other sites. While the minimum density found in Kalabsha (1713.7 x 106 unit m^{-2}).

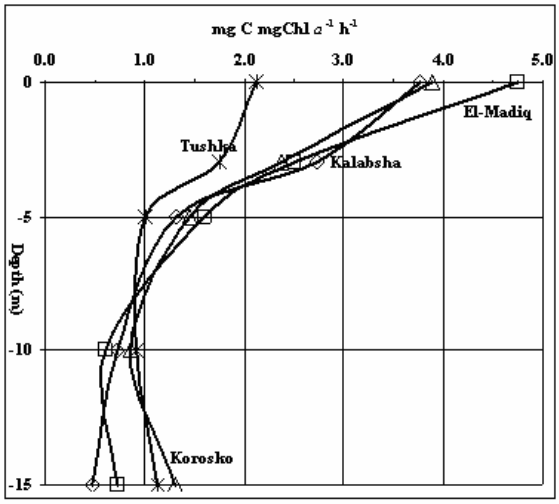

Figure 8. Depth profile of assimilation rate (mg C $mgChl^{-1}$ h^{-1}).

Qualitative investigation of phytoplankton showed that it was dominated by Cyanophyceae, where *Lyngbya limnetica*, *Oscillatoria limnetica*, *Oscillatoria planctonica* and *Microcystis aeruginosa* were the most common species. Chlorophyceae had more diversity than the others did, and dominated by *Chlorella vulgaris* and *Ankistrodesmus falcatus*. Bacillariophyceae were low in both diversity and density, whereas *Cyclotella ocellata* was the most frequent species. Dinophyceae was scarce and could not be observed in some locations.

4. Discussion

Lake Nasser is a newly created headwater of Nile River after construction of the High Dam (1968) at Aswan City (Egypt). It had not yet reached the steady state. About 79 % of the total lake surface area (4900 km^2) is employments, outside the main valley, and locally named Khors. It contained only 86.4 km^3 of water, forming about 55% of the total lake volume (Entz and Latif, 1974). Ecological status of water body in Khors is different than those characterize the main channel. Each one has specific environmental conditions. Therefore, its local variations in the limnological characteristics are expected to be of considerable importance.

Secchi disc readings have been occasionally used to deduce vertical extinction coefficient and euphotic zone depth, but in general such calculations are uncertain, particularly when clear and turbid waters are compared (Lund and Talling, 1957). That is manly controlled by suspended organic or/and inorganic maters. In the present study, water visibility in khors were mainly biologically controlled because it was reversely associated with integrated phytoplankton density in that water column (r = 0.75). It increased downstream with a maximum of 3.15 m in Kalabsha. In comparison with the previous investigations, Abd El-Monem (1995) found that water turbidity at the northern region of the main channel (down stream) are biologically controlled, while in the southern side it was affected with the suspended inorganic matters carried with the flooded water.

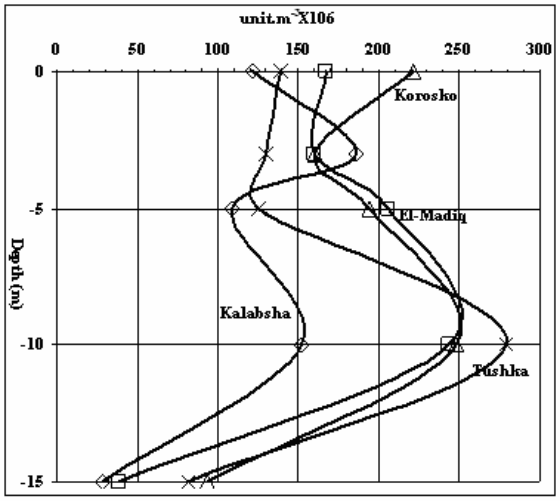

Figure 9. Depth profile of phytoplankton density (unit m^{-3} x 10^6).

In wintertime, there is a complete upwelling in the water body, and the lake lies under unstratified conditions. But during summer, water column was thermoclined and temperature of water body was increased upstream. In the upper layer, water temperature was rising up to 31.8 °C. Difference in water temperature (amplitude) in 15 m layer varied from 1.4 °C in Kalabsha to 5.3 °C in Toshka. Its amplitude was increased upstream, but it can extend more down to 15 m (studied zone) in the northern khors. This agrees with Abdel Rahman and Goma (1993). On this concept, Entz and Latif (1974) who surveyed the lake during the filling phase (1972-1973), reported that the time of total circulation is ending in March or April when the stratification started to develop. The depth of metalimnion is usually initiated in 12-17 m depth with a peculiar decline to the south. So the epilimnion is usually shallow in the north section and becomes deeper towards the inflow of Nile River water at the south. Under stratified conditions, temperature of the epilimnion is rising up to 25-29 °C. Higher temperatures are unusual, except on very calm days when within the upper 1 or 1.5 m the temperature can rise up to 30 °C. This may be caused by the decrease of thermal stability of the lake towards the south and the inflowing flood water. Stratification is usually dissolved from the south, starting in September and completed gradually towards Aswan in November.

Spatial distribution of DO along the water column showed that Oxygen distribution has a similar trend as that of the temperature. So, water depth up to 10 m was well oxygenated and represented the epilimnion layer while metalimnion can be extended to 15 m depth and hypolimnion layers initiated down. The value of hypolimnion was the widest near the High Dam; and is

diminishing to the south. Concerning yearly cycling of DO, Entz and Latif (1974) found that water is saturated till the bottom during winter circulation. From March, when the enormous development of the phytoplankton starts, there is a strong increase in DO content within the surface layer up to 14-18 mgO_2 l^{-1}. But, because of the increased thermal stability, the metalimnion is formed, and the hypolimnion becomes poorer and poorer in oxygen. The gradient of DO in metalimnion layer, which occurs when the hypolimnion becomes severely deoxygenated, creates a chemically diverse layer. The main loss of oxygen in that layer is probably due to decomposing sinking plankton organisms forming the so called plankton rain. The bacterial activity might be the most vivid just below the metalimnion.

In warm tropical water, small temperature differences have disproportionately large effects on the density of consumers and hence on the stability of stratification (Talling, 1957). This study reveals that the upper 10 m layer represents the critical zone in physical, chemical, and biological activities along the water column during summer. In spite of a limited decrease in water temperature, that depth included a noticeable variation in phytoplankton activity among the water column. It included the peak of phytoplankton density, biomass (Chl *a*) and phaeopigments. As well as it is still well oxygenated. Those parameters sharply declined downward.

In aquatic ecosystem, phytoplankton density, biomass, and its photosynthetic capacity may indicate eutrophic status of the lake. This study showed that depth profiles of phytoplankton standing crops are associated with water thermal stratification. Phytoplankton density is much greater in epilimnion than hypolimnion. Epilimnion was turbulent, and phytoplankters are maintained in suspension in it. Reynolds (1984) established that many cellular processes of phytoplankton are temperature-dependent, especially between 25 and 40 ^{0}C. Other physical and chemical factors, as well as biological (heterotrophic organisms), can affect the temporal distribution of phytoplankton in the lakes' water. Grazer and predator populations may control phytoplankton population directly and indirectly by large zooplankton, which can transport significant quantity of nutrients from the epilimnion layer (Longhurst and Harrison, 1988). So Regional variation of phytoplankton reflects its response to varying environmental conditions; and is affected by advection, turbulence, and grazing (Chang and Bradford, 1985).

Spatial and vertical distributions of net primary productivity in the lake water are varied and reflected the response of phytoplankton to ecological variables. Its values were limited between 31.9 and 74.4 mgC m^{-3} h^{-1}. Trend of its fluctuations in water column was irregular. Maximum production value was detected in surface water at El-Madiq and Korosko while it was at 3 and 10 m at kalabsha and toshka, respectively. Integrated column productivity for the studies depths revealed that korosko was the highest productive area (947.8 mgC m^{-2} h^{-1}), while El-Madiq was the lowest one (713.9 mgC m^{-2} h^{-1}). It was correlated with that equivalent concentration of Chl *a*. Tilzer (1983) reported that light fractions absorbed by algae increase with biomass but decreases with rising inorganic turbidity.

Generally, variability of primary productivity in the given results cannot be related to specific factor/s. There may be a variety of factors controlling primary productivity, mostly abiotic in nature such as light climate, turbulence, and nutrients (Tilzer, 1989). It was mainly limited by light penetration (Secchi depth) and turbidity when nutrients are in excess of growth demands. Nevertheless, primary productivity control by the food web has been shown to be sometimes as influential as the aforementioned abiotic factors (Carpenter et al., 1987). Water movement can result in marked variations in interception of light by phytoplankton (Grobbelaar and Stegmann, 1976). One therefore has to pay attention to other biotic components of the lacustrine ecosystem if a clear picture of the primary productivity is to be achieved. This is especially true for hypertrophic ecosystem where physical features are suspected to control primary productivity in view of nutrient excess (Robarts, 1984). Extensive human land use results in various levels of impact on actual net primary production. In a few regions, such as the Nile valley, irrigation has resulted in a considerable increase in primary production (Haberl, 2007).

Lake Nasser is one of the main sources of fishing in Egypt. Variability of the entire studied parameters, in the water budget during summer stratification, are greatly affected on the seasonal distribution of fishes, and can be controlled on fisheries and fishing activities during the whole year. In addition, the expected fish yield and its sustainability can be expected from the primary productivity of the lake. Because Entz and Latif (1974) mentioned that usually fish avoid water layer containing less than 2 or 3 mgO_2 l^{-1} and the same is true for fish food organisms. So fish searching for food usually avoid hypolimnion, and choose the epilimnion for a habitat. But they even don't like that, if the water temperature increases very quickly. So, most of them avoid the upper 1-2 m, and thus most of the fish may be located in summer time (during the stagnation period). Of course, there are typical littoral fish species which come likely to the very shallow water near the shore and find their best conditions for feeding. There in wintertime, the fish spread all over the lake water.

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Concentrations of Airborne Fungal Contamination in the Medical Surgery Operation Theaters (OT) of Different Hospitals in Northern Jordan

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Abstract

Total count and diversity of the airborne yeasts and filamentous fungi in the medical surgery operation theatres (OT) of six hospitals in northern Jordan were investigated. Sixty five air samples of 100 liters volume/min were collected by a microbiological air sampler from these units during the period June-Decemeber/2005. Air samples were impacted on Sabouraud Dextrose Agar (SDA), and then incubated at 25 °C for 21 days. All fungal colonies appeared on agar plates were sub-cultured on two SDA plates, after that incubated at 37 °C and 25 °C for 48 h and 21 days, respectively, and then identified based on the microscopic colony morphology and germ tube test. The average fungal count ranged between 88 and 259 CFU/m^3 with the highest count observed in hospital E (259 CFU/m^3), and the lowest in hospital A (31 CFU/m^3). More diverse colonies (2-13) were observed during June-September than after September (2-3) with the highest diversity (13 colonies) in hospital E. *Aspergillus* spp. (*A. fumigatus*, *A. niger*, *A. flavus*, *A. glaucus* and *A. terreus*) and other molds (*Penicillium* spp., *Mucor* spp., *Rhizopus* spp, *Graphium* spp, *Geotrichum* spp, *Trichophyton* spp, *Scopulariopsis* spp, *Fusarium* spp and *Microsporum* spp) were identified. Two types of yeasts were identified as *Candida* spp. and *Blastomyces* spp.

الملخص

تم في هذه الدراسة تحديد العدد الكلي وتنوع الفطريات و الخمائر المنتقلة بواسطة الهواء في غرف العمليات لستة مستشفيات في مدينة اربد. جمعت 65 عينة هواء (100 لتر/دقيقة) بواسطة جهاز خاص بذلك للفترة مابين شهر حزيران إلى شهر كانون الثاني/2005، وذلك باستخدام أطباق الوسط الزراعي SDA والتي حفظت على درجة حرارة 25 مئوي لمدة 21 يوم. تم إعادة تنمية كل مستعمرة فطرية ظهرت على هذه الأطباق على طبقين من وسط SDA وحفظها على درجة حرارة 37 و 25 مئوي لمدة يومين و 21 يوم على التوالي ومن ثم شخصت هذه الفطريات بناء على الشكل ألمجهري وفحص أنبوب الإنماء. تراوح عدد الفطريات ما بين 88-259 CFU/m^3 وبملاحظة أعلى عدد في مستشفى E (259 CFU/m^3) وأقل عدد في مستشفى A (31 CFU/m^3). كان التنوع أكثر في أشهر حزيران-أيلول (2-13) عما عليه في بقية مدة الدراسة (2-3) وملاحظة أكثر تنوعا (13 مستعمرة) في مستشفى E. تم عزل وتشخيص العديد من الفطريات مثل *Aspergillus* spp. (A. *fumigatus*, *A. niger*, *A. flavus*, *A. glaucus*, *A.terreus*) وفطريات أخرى مثل (*Penicillium* spp., *Mucor* spp., *Rhizopus* spp, *Graphium* spp, *Geotrichum* spp, *Trichophyton* spp, *Scopulariopsis* spp, *Fusarium* spp and *Microsporum* spp) ونوعين من الخمائر هي *Candida* spp. و *Blastomyces* spp.

KeyWords: Airborne; Fungi; Jordan; Hospitals; Operating Theaters.

1. Introduction

The presence of high concentrations of airborne microorganisms within the indoor environments is of increasing concern with respect to many acute diseases, infections, and allergies (Lugauska and Krikstaponis, 2004), and it is an indication of degree of cleanliness of these environments. Indoor environment, of which hospitals is of particular concern, contains different types of microorganisms (Saad, 2003), thus patients may serve as a source of pathogenic microbes to other patients, staff, and hospital visitors.

Bio-aerosols, of which fungal spores are one of the major types of microorganisms, cab be present in all hospital environments, and may be transmitted through air, outdoor air, visitors, patients, and air conditions (Beggs, 2003; Manuel and Kibbler, 1998). The evaluation of bacterial count, types, and diversity in hospitals rooms, especially in sensitive units like medical surgery operation theatres (OT) has raised worldwide concern. Approximately 10% of all patient infections are suspected

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to be hospital-acquired (Meers et al., 1990). These infections can have serious consequences in terms of increased patient mortality, morbidity, and length of hospital stay and overall costs.

This study investigates the measurements of airborne concentrations of fungi in the medical surgery operation theaters (OT) of six different hospitals in northern Jordan. Isolation and identification of airborne fungal genera and/or species being impacted from the indoor environment of OT of these hospitals are reported.

2. Materials and Methods

2.1. Hospitals Visited

The study was carried out looking for airborne fungi in the medical surgery operation theatres (OT) for six different hospitals serving a population of about 1.3 million in Irbid and its providence, Northern Jordan (Table 1).

Table 1: Numbers and diversity of isolated fungi/m³ during air samplings in the atmosphere at the medical surgery operation theaters (OT) of six different hospitals in Northern Jordan during June-August and September to December.

Hospital			First period of study (June to August)		
	No. of beds	Date Established	No. of Air Samples	Range No. of Colonies/Diversity of Colonies	Average No. of Colonies/ Diversity of Colonies
A	850	2002	10	0-80/0-4	31*,**/2
B	150	1985	6	45-340/4-12	137 * /9
C	100	2000	3	0-180/0-5	84 *,** /3
D	100	1992	7	15-70/2-5	43 *,** /4
E	350	1973	10	70-720/5-22	259 *,** /13
F	150	1988	4	5-60/1-6	38 * /4
Total No. of Air Samples			40		
Total No. of Colonies (%)			112 (42.1%)		
% of *C. albicans* from total isolates			3.6		
Hospital			Second period of study (September to December)		
	No. of beds	Date Established	No. of Air Samples	Range No. of Colonies/Diversity of Colonies	Average No. of Colonies/ Diversity of Colonies
A	850	2002	4	40-110/1-4	80*/2
B	150	1985	3	50-100/2	80*/2
C	100	2000	4	30-180/1-4	118*/3
D	100	1992	4	40-200/2-6	103*/3
E	350	1973	6	90-220/2-4	133*/3
F	150	1988	4	100-210/2-3	148*/2
Total No. of Air Samples			25		
Total No. of Colonies (%)			154 (57.9%)		
% of *C. albicans* from total isolates			2.0		

* Denote no significant differences between hospitals

** Denote significant differences between hospitals when hospital E is compared to A, C and D hospitals ($P \leq 0.05$)

2.2. Air Sampling

Between June and December (2005), a total of 65 air samplings were performed in the internal atmosphere of OT units. Each air sampling was performed using a special device; Microbiological Air Sampler (M.A.Q.S.II-90)/ OXOID, UK). That device can hold 90 mm Petri dishes containing sabouraud dextrose agar (SDA) within an autoclavable anodized aluminum head of 380 holes. The sampler was set at an air-sampling rate of 100 l /min for two minutes per sample. During each visit, duplicate air samples were collected with sampling made at one meter elevation from the OT floor (i.e. at the same level of the patient's bed). The anodized aluminum head of the microbiological air sampler between the duplicate air samples collection was sterilized by 70% alcohol using a sterile cotton swab. All samplings were made when the OT was not in use, i.e. at a period of idling for next operation.

2.3. Sample Processing

After impacting the air borne microbial samples on SDA (Oxoid, UK), they were transported to laboratory and immediately incubated at 25° C with daily observation of the plates for fungal growth up to 21 days. Counts of different fungal growths wre coded as they appeared on SDA plates and were designated to thier specific genus and species, and later were recorded by using the colony counter (560, Suntex, Labolan).

2.4. Fungal Identification

During incubation period, different fungal colonies were subjected to macroscopic and microscopic examination to observe their growth, nature of their

mycelium, and hyphae structure. Filamentous fungal growth - as mold and /or yeast- were present on SDA media plates; and were sub-cultured on two separate SDA culture plates. One plate was incubated at 37° C, and the second was incubated at 25° C (Koneman *et al*., 1997). Pure culture growth of each mold and/or yeast colony appeared on those plates; and was examined under magnification for their microscopic structures and cross identified, by using mycological keys manuals and textbooks. Direct wet mounts in lacto-phenol cotton blue (LPCB) and microculture slides preparation were made to determine the nature of the fungal hyphae and the fructifications, such as conidiophores, conidia production. And the kind of conidia produced by the molds was loosely examined.

Yeast colonies were determined whether being colonies of *Candida albicans* or not, according to procedure outlined by Larone (1987). This test was performed by transferring a loop-full from single colony growth of yeast on the SGA plates to 0.5 ml of sterile serum in a test tube; and incubated the tubes at 35-37°C for approximately three hours. The yeast-serum culture examined as wet mount under magnification (X400) for the formation of germ tube (psuedohyphae) which is an indication of positive identification of *Candida albicans* (Larone, 1987).

2.5. 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 $\alpha = 0.05$.

3. Results

From June to December-2005, a total of 65 air samplings were made from the atmosphere of medical surgery operation theaters (OT) of six hospitals in Irbid city, Northern Jordan (Table 1). A total of 266 fungal colonies were isolated and recognized as 112 colonies (42.1%) appeared during June to August (Table 1). Molds comprised 45.5% while yeasts comprised 54.5% (data not shown). However, 154 colonies (57.9%) (Table 1) were recognized during September to December of which 41% were molds and 59% were yeasts (data not shown).

Fungal counts in OT of the first period of study ranged between 31 and 259 CFU/m³. However, fungal counts in the second period of study ranged between 80 and 148 CFU/m³. Data showed that fungal counts in OT during the second period were higher than the first period with the exception of hospitals B and E (Table 1). This variation was not clear in hospitals A and C and during the same periods. When the fungal count in the different hospitals was compared between the two periods, data indicated that hospitals C, D and F exhibited higher counts during Autumn than Summer (Table 1). In general there is a significant difference in total fungal count between hospitals E and A, B, D and F hospitals ($P < 0.05$).

Fungal diversity in the first period of study ranged between 2 and 13 different colonies. However, fungal diversity in the second period of study ranged between 2 and 3 different colonies. Data revealed that fungal diversity in the OT during Summer was higher than Autumn and particularly in hospitals B and E (Table 1). This variation was not clear in hospitals A and C and during the same periods.

During both periods, filamentous fungi were distributed at considerable levels in all visited hospitals with variation among different species of isolated fungi. Aspergilla; such as *A. fumigatus*, *A. niger, A. flavus, A. glaucus* and *A. terreus* constituted 78-85% / 71-80% of the isolated fungi (data not shown). The other proportion (15-22% / 20-29%) of isolated molds was identified as *Penicillium* spp, *Mucor*, *Rhizopus*, *Graphium*, *Geotrichum*, *Trichophyton*, *Scopulariopsis*, *Fusarium* and *Microsporum* (data not shown).

Distribution of airborne yeasts was different during the two periods of study with *Candida* spp. (76%) and *Blastomyces* spp. (15%) being recognized during June to August (data not shown). However, during September to December for the above yeasts, this distribution was 78% and 4%, respectively (data not shown). *Candida albicans* encountered 3.6% and 2% of total fungal isolates during Summer and Autumn, respectively (Table 1).

4. Discussion

Hospital environments are complex environments because they contain different types of microorganisms. Airborne microorganisms are one of these microbes and their presence, numbers, and types can indicate the degree of cleanliness of these environments. There are wide varieties of factors which influence airborne counts, and therefore influence hospital infection rates (Jaffal et al., 1997; WHO, 2002).

Fungi and bacteria are the major types of microorganisms present in all hospital environments that may be transmitted through air, outdoor air, visitors, patients, and air conditions. These are the major sources of hospitals indoor contamination (Beggs, 2003; Manuel and Kibbler, 1998). The level and diversity of biocontamination in hospitals environments depend on different factors such as the number and activities of visitors, patient, design of hospitals rooms, disinfectant process and methods, outdoor air and dust, and other factors (Sessa et al., 2002; Saad, 2003). The evaluation of count, types, and diversity of biocontamination in hospitals rooms especially OT is very important to control and prevent hospital acquired infections (HAI).

Results showed that fungal counts in all studied hospitals units during Autumn are higher than during Summer. The higher number of fungi in hospitals in these seasons may be related to occupant density, temperature, and level of humidity. In addition, the bioaerosols containing microorganisms may reside in Autumn for long time in air than Summer. These results are consistent with those reported by Hou and Li (2003).

The fungal counts in OT of hospital E were higher in Summer than in Autumn, because the samples were collected after the process of disinfection and sterilization of these units in this hospital. The quantitative study of OT showed that the number of microorganisms in OT was considerably low. This is due to the high sanitary standards in OT, as compared to other hospital areas. The old design of hospitals B, D and E in comparison with hospital A that has been established in 2002 is the major reasons of the high level contamination. However the high level of

contamination in hospital F may be due to outdoor contamination.

Diversity of fungi is usually related to the count (Spengler and Saxton, 1983; Flannigan, 1992). Data showed that more fungi were isolated in Autumn than in Summer. The results are consistent with Hou and Li (2003) and Klanova and Hollerova (2003).

Studying airborne fungal spores is important to understand dissemination, spread, and movement of the microbes, particularly the pathogenic ones in the atmosphere (Moustafa and Kamel, 1976). During Autumn, the percentage of yeast increased while mold decreased in Summer. These results may be correlated with high level of humidity in Autumn than in Summer (Beggs, 2003). The common genera of fungi that are frequently isolated from the hospitals air are *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Graphium*, *Geotrichum*, *Trichophyton*, *Scopulariopsis*, *Fusarium* and *Microsporum* spp. However, the common genera of yeasts that are frequently isolated from hospitals are *Candida* spp. and *Blastomyces* spp. (Lugauska and Krikstaponis, 2004). Significant numbers of *Aspergillus* spp. (71-85%) and *C. albicans* (35.5-37%) were shown in comparison with other fungal species. *Aspergillus* spp. and *C. albicans* as reported in different studies (Ahmad et al., 2003; Weinberger et al., 1997; Overberger et al. 1995; Harvey and Hyers, 1987); and were considered as the major source of hospital fungal infections. Manuel and Kibbler (1998). Overberger et al. (1995) found that 70-80% of the fungi in hospitals air were *Aspergillus* spp. This study showed similar distribution of the mold and yeast species.

5. Conclusion

In conclusion, airborne concentrations of fungi in the operating theaters (OT) of six different hospitals in northern Jordan indicated higher counts but less divers during autumn than during summer. The high sanitary standards in OT units and new design of hospitals are the major reasons of the low level of contamination.

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