مجلة المختار للعلوم
AL-Mukhtar Journal of Sciences

Volume: 33 Issue: 2 2018

ISSN:26-17-2178 (Print) ISSN:26-17-2186 (Online)

دار الكتب الوطنية - رقم الإيداع القانوني 280-2013

مجلة المختار للعلوم

جامعة عمر المختار

البيضاء، ليبيا

مجلة علمية محكّمة، المجلد الثالث والثلاثون، العدد الثاني، 2018

تصدر عن جامعة عمر المختار، البيضاء، ليبيا.

مجلة المختار للعلوم

رقم الايداع في المكتبة الوطنية 2013/280/بنغازي

جامعة عمر المختار – البيضاء – ليبيا

مجلة محّكمة تصدر عن جامعة عمر المختار، البيضاء، ليبيا

مجلة علمية محكّمة، المجلد الثالث والثلاثون، العدد الثاني، 2018

بريد إلكتروني: omu.j.sci@omu.edu.ly

ص.ب. 919 البيضاء – ليبيا، فاكس: 7053 463 69 218+

أعضاء هيئة التحرير

مجلة المختار للعلوم 33:(2)، 2018

Al-Mukhtar Journal of Sciences33: (2), 2018

Al-Mukhtar Journal of Sciences 33 (2): 78-89, 2018

Effect of Incubation Periods, Medium Volumes and Explants Density on *in Vitro* Shoot Formation and Growth and Cost of Multiplication of Moris Pineapple (*Ananas comosus* L.)

Abdelhamid A. Hamad
Department of Horticulture, Faculty of Agriculture, Omar Al-Mukhtar University, Al-Bayda, Libya

Received: 9 February 2017 / Accepted: 20 April 2017
Doi: https://doi.org/10.54172/mjsc.v33i2.290

Abstract: This study was conducted to investigate the effect of combinations of four incubation periods (30, 45, 60 and 75 days), three explants density (one, two and three) and four volumes of medium (3, 6, 9 and 12 ml) in the in vitro shoot formation per explant, total shoots and total cost per liter of medium and cost per shoot of Moris pineapple. The highest shoot per explant was obtained in combination of three explants in 12 ml of medium incubated for 60 days, three explants in 9 and in 12 ml of medium and one explant in 3 and in 9 ml of medium incubated for 75 days but at different total shoots (2750, 3667, 2750, 3667 and 1222 shoots), total cost (USA $25.85, 36.09, 28.39, 95.32 and 36.06) per liter of medium. Combinations in which three explants were used per culture resulted in lowest cost per shoot (1.0 cent) while combination in which one explant was used per culture, the cost per shoot was two to three times higher (2.6 and 2.9 cents). Combination of three explants, 3 ml of medium and 75 days of incubation, on the other hand, resulted in formation of only 6 shoots per explant (almost half of the highest obtained rate) but in highest total shoot per liter of medium (6000 shoots) at highest total cost (USA $95.32) and intermediate cost per shoot (1.6 cents). Combination of three explants, 6 ml of medium and 75 days resulted in formation of 10 shoots per explant, production of 5000 shoots per liter of medium at lowest cost per shoot (1.0 cent) and intermediate total cost per liter of medium (USA $ 50.68). Using of one explant in 12 ml for 30 days resulted in formation of 5 shoots per explant, highest cost per shoot (5.0 cents) and lowest total shoots (417 shoots) and lowest total cost (USA $ 20.73) per liter of medium.

Keywords: Total shoots; Total cost; Medium volume; Explants density; Incubation periods; Pineapple; *Ananas comosus.*

INTRODUCTION

A single multiplication cycle of pineapple ranged from a minimum of 15 days (Firoozabady & Gutterson, 2003) to a maximum of 112 days (Nelson et al., 2015) in which explants placed in as low as 10 (Hamad & Taha, 2003) and as high as 100 ml (Fernando, 1986) of MS medium. Yet 30 and 45 days and 20 and 25 ml of solid (Sripaoraya et al., 2003; Sunitibala Devi et al., 1997) and liquid medium (Be & Debergh, 2006; Pérez et al., 2009; Pérez et al., 2012; Teixeira et al., 2006) are the most commonly used cycle length and medium volume for *in vitro* shoot multiplication. Explants were used at density of 1(A. M. Hamad & R. Taha, 2008; A. M. Hamad & R. M. Taha, 2008a, 2008b), 2 (Be & Debergh, 2006; Soneji et al., 2002), 3 (Hamad & Taha, 2003, 2009), 4 (Khan et al., 2004), 5 (Daquinta et al., 1997; Pérez et al., 2009; Pérez et al., 2012), 8 (Dal Vesco et al., 2001), and 10 (Zuraida et al., 2011) explants per culture. Explants could be used intact (A. M. Hamad & R. Taha, 2008; Khan et al., 2004), halved single shoot

***Corresponding Author:** Abdelhamid M. Hamad,abdelhamidhamad@gmail.com , Faculty of Agriculture, Omar Al-Mukhtar University El-Bayda, Libya.

(Hamad, 2017,(Almeida et al., 2002; Bhatia & Ashwath, 2002) and cluster of shoots (Escalona et al., 1999; Firoozabady & Gutterson, 2003; Hamad & Taha, 2009). Optimal hormone treatment varied at different incubation periods (A. M. Hamad & R. M. Taha, 2008b; Kofi & Adachi, 1993). The focus on these studies was mainly on testing hormone types, concentrations, and combinations and on comparing of solid and liquid media at fixed level of other factors and the assessment was based on the shoot formation rate and total shoot per one cycle (Escalona et al., 1999) and several cycles of multiplication (A. M. Hamad & R. Taha, 2008). Investigation of different combinations of explants density, volume of medium per culture, and incubation periods are not only important for their expected effect on shoot formation per explant but also on the possibly obtainable total shoots per liter of medium. The objective of this study was to investigate the effect of three explant densities (one, two and three explants per culture) and 4 volumes of medium (3, 6, 9 and 12 ml per culture) and four incubation periods (30, 45, 60 and 75 days) on the *in vitro* shoot formation per explants and on total shoots and cost per one liter of medium and cost per shoot of Moris pineapple.

MATERIALS AND METHODS

Full strength MS(Murashige & Skoog, 1962) medium enriched with sucrose at 20 g/l and BAP at 2.0 mg/l was adjusted to pH 5.0, sterilized at 121 ^{0}C and 1.5 kg /cm^2 for 25 minutes and dispensed under laminar air flow cabinet into 144 culture tubes. Each 36 of the 144 cultures tubes received 3, 6, 9 and 12 ml of the medium. One, two and three 15 to 20 mm long shoots were taken from 6-months old Moris stock cultures and placed in each 9 of the 36 culture tubes that received an equal volume of medium. The cultures incubated under constant temperature 25 ^{0}C and 16 hours of light provided by cool white fluorescent lamps. After 30 days of incubation, 9 cultures from each combination of explants density and volumes of medium were randomly picked and used for data collection. The multiple shoots complex of each explant was picked out, placed over a squared paper and separated into individual shoots for counting and measuring the length of shoots.

Data were used to establish two tables, one for total shoots and one for total shoot length per culture after 30 days incubation period. The table of total shoots per culture was converted to table of shoot formation per explant by dividing by the number of explants per culture tube and to table of total shoot per liter of medium by multiplying by 1000 and dividing by the medium volume per culture. Table of total shoot length was converted to table of shoot length per explant by dividing by the total shoots per culture. Each of these tables is two factors table (explants and volumes). After 45, 60 and 75 days of incubation, the same procedures were repeated to establish tables for total shoots and total shoots length per culture and shoots formation and shoot length per explant after 45, 60 and 75 days of incubation. The two factors tables (explants, volumes) of the same parameters (shoot formation, shoot length per explant and total shoots per liter of medium) at each incubation period were combined to obtain three factors tables (explants, volumes, and incubation) and the data were subjected to analysis of variance and separation of means by Duncan Multiple Range Test (Duncan, 1955) at $p \leq 0.05$ using SPSS statistical package No 11. Table of total shoots per liter of medium was used for estimation of total cost and cost per shoot.

RESULTS

Table 1 showed that five different combinations of explants density and medium volumes per culture and incubation periods resulted in highest shoot formation per explant (11 shoots). One of these combinations made of three explants per culture incubated in 12 ml of medium for 60 days. In the other four

ISSN: online 2617-2186 print 2617-2178

combinations, the incubation period was 75 days but of a different combination of explants density and medium volume per culture. One explant in 3 and 9 ml and three explants in 9 and 12 ml of medium. In all of these five combinations, the incubation period was longer than 45 days and none included density of two explants per culture. On the other hand, six different combinations resulted in the lowest shoot formation per explant (3 shoots).

In these combinations, the incubation was shorter than 60 days, the medium less than 9 ml, and the explants density was more than one explant per culture. At a fixed density of one explant, if the medium dispensed at 3 ml per culture, each 15 days increase in the incubation (30, 45, 60 and 75 days) increased the shoot formation per explant (4, 8, 9 and 11 shoots). However, if the medium dispensed at 6 ml, incubation for 45 days resulted in more shoots per explant (6 shoots) than incubation for 30 days (4 shoots) but equal to that obtained after 60 days (6 shoots). Extending the incubation to 75 days increased the shoot formation to 8 shoots. When the medium was dispensed at 9 ml, incubation for 30, 45 and 60 days resulted in equal shoots formation per explant (8 shoots) and extending the incubation to 75 days increased the shoots formation to 11 shoots per explant. In cultures containing 12 ml of medium, increasing the incubation from 30 to 45 and 60 days increased the shoots formation from 5 to 6 and 9 shoots per explants.

Extending the incubation to 75 days did not increase the shoots formation resulted in equal shoot formation as that of 60 days incubation (9 shoots). At density of one, the highest shoot formation (11) obtained when medium dispensed at 3 and 9 ml and culture incubated for 75 days. At fixed density of two explants, dispensing the medium at 3 ml per culture and incubation for 30 and 45 days resulted in equal shoots formation per explant (3 shoots) and increasing the incubation to 60 and 75 days increased the shoots formation to 4 and 6 shoots per explant respectively. If the medium dispensed at 6 ml, incubation for 30, 45 and 60 days resulted in equal shoots formation per explant (4 shoots) and increasing the incubation to 75 days increased the shoots formation to 5 shoots per explant while if medium dispensed at 9 ml, increasing the incubation from 30, 45 to 60 days increased shoot formation from 3 to 5 and 6 shoots but extending the incubation to 75 days did not increase the shoots formation more than that obtained after 60 days (6 shoots).

Dispensing the medium at 12 ml and incubation for 45 and 60 day resulted in equal shoot formation (5 shoots) and both were more than the shoot formation (3 shoots) after 30 days of incubation. Extending the incubation to 75 days doubled the shoot formation (9 shoots) compared to that obtained after 45 and 60 days of incubation. At a density of two explants, the highest shoot formation (9 shoots) obtained when medium dispensed at 12 ml and incubated for 75 days. Similar, the response of explants when three explants were used per culture were also varied depending on the volume of medium and incubation periods. When the medium dispensed at 6 and 9 ml per culture, each 15 days increase in the incubation (30, 45, 60 and 75 days) increased the shoot formation per explant (3, 4, 6 and 10 shoots) and (5, 6, 7 and 11 shoots) respectively. However, when the medium dispensed at 3 ml, incubation for 30 and 45 days resulted in equal shoots formation per explant (3 shoots) and increasing the incubation to 60 and 75 days increased the shoots formation to 4 and 6 shoots per explant respectively. If the medium dispensed at 12 ml per culture, increasing the incubation from 30 to 45 and 60 days increased the shoots formation from 5 to 7 and 11 shoots per explant, but extending the incubation to 75 days did not increase the shoots formation more than that obtained after 60 days (11 shoots).

At a density of three, the highest shoot for-

ISSN: online 2617-2186 print 2617-2178

mation (11) obtained when medium dispensed at 9 ml and incubated for 75 days and when medium dispensed at 12 ml and incubated for 60 days. Converting the shoot formation per explant to total shoot production per liter of medium (Table, 2) showed that the five combinations (3 explants in 12 ml and incubation for 60 days and 3 explants in 9 and 12 ml and one explants in 3 and 9 ml and incubation for 75 days) which resulted in the highest shoot formation per explant (11 shoots) resulted in different total shoots per liter of medium (2750, 3667, 2750, 3667 and 1222 shoots). Three of these combinations (three explants in 9 and 12 ml of medium for 75 days and three explants in 12 ml of medium for 60 days) resulted in lowest cost per shoot 0.1 cent and lowest total cost (USA $ 25.85; 36.09; 28.39) while the cost per shoot of the other two combinations was three-time higher (2.6 and 2.9 cents) and the total cost was USA $ 95.32 and 36.09 (Table, 2).

On the contrary, combination in which the shoot formation was 5 shoots less than the highest obtainable shoot formation (three explants in 3 ml of medium for 75 days) resulted in the highest total shoots per liter (6000 shoots) but at highest total cost (USA $ 95.32) and the cost per shoot was two times (1.6 cent) the possible lowest cost per shoot (0.9 cent).

DISCUSSION

This study demonstrated that unless the effect of explants density, volume of medium and incubation period were tested at combinations of different levels, physiological studies of shoot formation and commercial protocol for *in vitro* multiplication could neither be developed nor verified. The results showed that changing the level of any one of these factors lead to a different optimum combination of the other two factor for highest shoot formation per explants (Table, 1), total shoots production and total cost per liter of medium and cost per shoot (Table, 2). Hence, to achieve the different goals for physiologist and propagators, dispensing of medium per culture should be made in accordance with the intended density of explants and incubation period and vice versa. According to shoot formation per explant, five different combinations of explants density, medium volume per culture and incubation periods (Table, 1) resulted in the highest shoot formation per explant (11 shoots). According to rates, either one of these five combinations could be recommended as the best treatment. However, Table 2 showed that higher shoot formation per explant may result in lower total shoots and lower shoot formation may result in higher total shoots per liter of medium. Combination of three explants, 3 ml and 75 days of incubation resulted in formation of only 6 shoots per explants (half of the highest obtained rate) but produced the highest total shoots (6000 shoots) while combination of one explant, 9 ml and 75 days of incubation resulted in highest shoot formation (11 shoots) but low total shoots (1222 shoots) per liter of medium. Which one is the more important than the other? The rate per explant or the total shoots per liter of medium depend on the researcher goal. Physiology judged by rate while for management and profitability of production a compromise between highest total shoots and lowest total cost per liter and cost per shoot are very crucial.

Two of the five combinations which resulted in the highest shoot formation (11 shoots) beside it resulted in different total shoots (3667 and 1222 shoots) and total cost (USA $ 95.32 and 36.09), the cost per shoot was 3 times higher (2.6 and 2.9 cents) than the lowest obtainable cost per shoot. The other three combinations resulted also in different total shoots (3667, 2750 and 2750 shoots) and total cost (USA $ 36.09; 28.39 and 25.85) per liter of medium (Table, 2) but in lowest cost per shoot (0.1 cent). In other words, if the combinations assessment was based on shoot formation per explant, there is a risk of recommending combination which produced shoots at three times higher cost per shoot

ISSN: online 2617-2186 print 2617-2178

(2.6 cents) than the possibly obtained lowest cost (0. 9 cent), and losing of possibly produced 2445 shoots (3667- 1222) and extra spending of USA $ 69.47 (95.32-25.85**)** per liter of medium.

On the other hand, if assessment was based on total shoots per liter of medium (Table, 2), combination of three explants, 3 ml and 75 days of incubation resulted in highest total shoots (6000 shoots), but in lower shoot formation per explant (6 shoots) and higher cost per shoot (1.6 cents) and highest total cost (USA $ 95.32) per liter of medium. That is, there is a risk of recommending combination which suppressed the shoot formation per explant, produced shoots at two times higher cost per shoot than the possibly obtained lowest cost (0. 9 cents) and at highest total cost (USA $ 95.32). The cost per shoot could be reduced to 0.1 cent, the total cost of USA $ 50.68 and the shoot formation increased to 10 shoots per explant if the medium dispensed at 6 instead of 3 ml, but the total shoots declined from 6000 to 5000 shoots per liter.

ISSN: online 2617-2186 print 2617-2178

Table (1). Effect of medium volumes, incubation periods and explants density per culture on the *in vitro* shoots formation per explant and shoot length of Moris pineapple.

MS	**Incubation periods (days) and explants density**											
(ml)	30			45			60			75		
	1	2	3	1	2	3	1	2	3	1	2	3
Shoots per explants												
3	4 e	3 e	3 e	8 bcde	3 e	3 e	9 bcd	4 e	4 e	11 b	6 cde	6 cde
6	4 e	4 e	3 e	6 cde	4 e	4 e	6 cde	4 e	6 cde	8 bcde	5 de	10 bc
9	8 bcde	3 e	5 de	8 bcde	5 de	6 cde	8 bcde	6 cde	7 bcde	11b	6 cde	11 b
12	5 de	3 e	5 de	6 cde	5 de	7 bcde	7 bcde	5 def	11 b	9 bcd	9 bcd	11 b
Shoot length (mm)												
3	8.7bcd	11.7abcd	9.3bcd	9.7bcd	11.7abcd	12.3abcd	10 bcd	13.7abcd	11.7abcd	10.7abcd	14.7 abc	15 ab
6	8.3 cd	8.3 cd	9.3bcd	10 bcd	9 bcd	8 d	11.3abcd	9.7 bcd	12.7abcd	14.7 abc	10.3 bcd	15 ab
9	7.3 d	7 d	7.3 d	7.7 d	7.7 d	8 d	9.3 bcd	12.3abcd	12.3abcd	12.7abcd	13.3abcd	13.7abc
12	8 d	7.7 d	7 d	9 bcd	9 bcd	8.7 bcd	9 bcd	11.7abcd	12 abcd	10 bcde	13.3abcd	16.7 a

Each combination of medium volume, explants density and incubation period consisted of nine culture tubes.
Explants (shoots) were cultured in culture tubes containing static liquid full strength MS medium enriched with sucrose at 20 g/ l and BAP at 2.0 mg/l and pH adjusted to 5.0 and incubated under constant temperature (25 ^{0} C) and 16 hours of light.
Means followed by same letters were not significantly different as tested by Duncan Multiple Range Test at $p \leq 0.05$.

ISSN: online 2617-2186 print 2617-2178

Table (2). Effect of medium volumes, incubation periods and explants density per culture on the total shoots and total cost per liter of medium and cost per shoot of Moris pineapple

MS	**Incubation periods (days) and explants density**											
(ml)	30			45			60			75		
	1	2	3	1	2	3	1	2	3	1	2	3
Total shoots per liter												
3	1333hij	2000efghi	3000cde	2667defg	2000efghi	3000cde	3000cde	2667defg	4000bc	3667cd	4000 bc	6000a
6	667 j	1333 hij	1500ghij	1000 ij	1333 hij	2000efghi	1000 ij	1333 hij	3000 cde	1333hij	1667fghi	5000b
9	889 ij	667 j	1667fghij	889 ij	1111 hij	2000efghi	889 ij	1333 hij	2333efgh	1222hij	1333 hi	3666cd
12	417 j	500 j	1250 hij	500 j	833 ij	1750fghij	583 j	833 ij	2750 def	750 j	1500ghi	2750def
Total cost per liter (USA $**)**												
3	64.58	64.58	64.58	74.83	74.83	74.83	85.07	85.07	85.07	95.32	95.32	95.3
6	35.27	35.27	35.27	40.41	40.41	40.41	45.54	45.54	45.54	50.68	50.68	50.68
9	25.85	25.85	25.85	29.26	29.26	29.26	32.68	32.68	32.68	36.09	36.09	36.09
12	20.73	20.73	20.73	23.29	23.29	23.29	25.84	25.84	25.84	28.39	28.39	28.39
Cost per shoot (USA $**)**												
3	0.048	0.032	0.022	0.028	0.037	0.025	0.028	0.032	0.021	0.026	0.024	0.016
6	0.053	0.026	0.023	0.040	0.030	0.020	0.045	0.034	0.015	0.038	0.030	0.010
9	0.029	0.039	0.015	0.033	0.026	0.015	0.037	0.024	0.014	0.029	0.027	0.010
12	0.050	0.041	0.016	0.046	0.028	0.013	0.044	0.031	0.009	0.038	0.019	0.010

Each combination of medium volume, explants density and incubation period consisted of nine culture tubes.
Explants (shoots) were cultured in culture tubes containing static liquid full strength MS medium enriched with sucrose at 20 gm/ l and BAP at 2.0 mg/l pH adjusted to 5.0 and incubated under constant temperature (25 ^{0} C) and 16 hours of light.
Means followed by same letters were not significantly different as tested by Duncan Multiple Range Test at $p \leq 0.05$.
Total shoots per liter computed by multiplying the average total shoots per culture tube by 1000 and dividing by the volume of medium per culture tube
Cost estimate included only the variable cost items (Medium, culture tubes, labor and electricity)

ISSN: online 2617-2186 print 2617-2178

That is losing of 1000 possibly produced shoots but saving of USA $ 44,64 per liter of medium. Similar, if one or two explants were used per culture, the highest total shoots per liter of the medium was (3667and 4000 shoots respectively) and the lowest cost per shoot in both cases (2.6 cents) obtained when medium dispensed at 3 ml per culture and incubated for 75 days. In a case when two explants were used, the cost per shoot could be reduced to 1.9 cents if the medium dispensed at 12 instead of 3 ml, but the expected total shoot per liter of medium declined from 4000 to 1500 shoots (Table, 2).

Tables 1 and 2 showed that using of explants at a density of one and two per culture did not serve the propagator goal of highest total shoots and lowest total cost per unit of medium. A density of three explants, on the other hand, could serve the propagator goal only if the medium dispensed at volume larger than 3 ml per culture and the incubation is longer than 45 days. Lowest cost per shoot is very crucial for both small and big companies while the production of highest total shoots is depending on the company budget, client demands, market size, and time of delivery. Depending on his budget and obligations, propagator could select the most suitable combination and decide the proper management of *in vitro* multiplication system.

The shoot formation at each fixed explants density depended on medium volumes, and at each fixed volume of medium depended on the explants density and on both cases on how long the cultures were incubated. Certain volumes of medium per culture and certain incubation periods are optimum for certain explants density and not for the others. Shoot formation per explant reflects how the different factors directly or via interaction with each other affect the process of shoot formation. At a fixed density of one explant and incubation of 30 days, the shoot formation increased as the volume of medium increased up to 9 ml per culture and declined at 12 ml (Table, 1). This decline in shoot formation if a volume of medium was higher than 9 ml indicated that the number of some ions of the medium nutrient or hormone content of the 12 ml reached inhibitory level. However, if one explant incubated for 45, 60 and 75 days, the decline of shoot formation started if the volume of medium was higher than 3 ml per culture. Using a larger volume of medium enhance the shoot formation in case of shorter incubation but retard the shoot formation in case of longer incubation. It seemed that the promotion effect of a larger volume of medium when explants incubated for a shorter period could be reversed by longer incubation.

Explants during the first 30 days of incubation might have inefficient nutrient uptake and require a larger volume of medium while after 30 days of incubation explant developed efficient nutrient uptake and absorbed enough nutrient even if the volume per culture was too low. In addition, after 30 days of incubation, the explants may release certain extracts that interact with certain components of the medium to produce an inhibitor of shoot formation. On the contrary, at a fixed density of three explants, the highest shoot formation at any of the incubation period obtained when medium dispensed at the largest volume of medium (12 ml) per culture. The shoot formation increased as the volume of medium per culture increased. This may indicate that presence of three explants would share the content and reduce the amount of certain medium components below its inhibitory level. At a density of one explant per culture, two different volumes of medium (3 and 9 ml) at same incubation period (75 days) could be used to induce one explant to express its highest shoot formation ability (11 shoots). At a density of three explants, also two different but larger volumes of medium (9 and 12 ml) at same incubation (75 days) and same medium volume (12 ml) but different incubation period (60 and 75 days) resulted in highest shoot formation. One of these volumes of

ISSN: online 2617-2186 print 2617-2178

medium (9 ml) and one of these incubation periods (75 days) is shared by the two different explants density. All of these different combinations of medium volume and incubation periods resulted in highest and equal shoot formation (11 shoots) whether the explant was one or three.

In case of two explants per culture, none of the combinations resulted in 11 shoots. But also, two different volumes of medium and two different incubation periods could be used to induce highest shoot formation per explant (9 shoots). Medium dispensed at 9 ml and incubation for 60 days and dispensed at 12 ml and incubation for 75 days. However, when the response of one and three explants was compared, it showed that depending on the volume of medium and incubation period, one explant per culture could result in higher, equal or less shoot formation rate than that of three explants per culture and vice versa (Table, 1). The effect of medium volume and explants density on shoot formation could be related to the shortage (competition) or oversupply (inhibition) of medium components. The effect of incubation period is a result of a complicated relationship of growth, changes of medium components and vessel atmosphere over time. If the cultures containing less than 12 ml and the incubation period less than 75 days, using of one explant resulted in higher rate of shoot formation than using three explants per culture. The low rate of shoot formation of the three explants may due to competition for nutrient or hormone content of the medium. On the contrary, if the culture contained 12 ml of medium irrespective of the incubation period (30, 45, 60 and 75 days), using of three explants resulted in higher rate of shoot formation per explant than using one explant per culture. The hormone or ions content of the larger volume of the medium reached an inhibitory level for the one explant and suppressed its shoot formation. However, irrespective of using smaller or larger volume of medium per culture if the culture incubated for 75 days, three explants resulted in higher rate of shoot formation than one explant. In this case, competition and over supply could not explain the result. Over longer incubation, the three explants might release an extract that overcomes shortage as well as the inhibitory effect of medium components and enhances the shoot formation of the three explants more than one explant. (Konan et al., 2007) reported that when more than one explants were used per culture, explants size-related cofactor control the *in vitro* rooting ability of oil palm. The higher shoot formation of the three explants than the one at certain medium volume and incubation and lower at other indicated presence of explants density- related cofactors. Promotion or inhibition nature of the cofactor depended on the volume of medium dispensed per culture and incubation period. Elucidation the role of medium volume, explants density and incubation period in the shoot formation process require future investigation using specifically selected combinations and retesting their effect using medium of different strength and pH adjustments in connection with a chemical analysis of medium and explants and histological investigation over incubation.

REFERENCES

Almeida, W. A. B. D., Santana, G. S., Rodriguez, A. P., & Costa, M. A. P. D. C. (2002). Optimization of a protocol for the micropropagation of pineapple. *Revista Brasileira de Fruticultura, 24*(2), 296-300.

Be, L., & Debergh, P. (2006). Potential low-cost micropropagation of pineapple (Ananas comosus). *South African Journal of Botany, 72*(2), 191-194.

Bhatia, P., & Ashwath, N. (2002). Development of a rapid method for micropropagation of a new pineapple [Ananas comosus (l.) Murr.] clone,'Yeppoon gold'. International

ISSN: online 2617-2186 print 2617-2178

Symposium on Tropical and Subtropical Fruits 575,

Dal Vesco, L. L., de Almeida Pinto, A., Zaffari, G. R., Nodari, R. O., dos Reis, M. S., & Guerra, M. P. (2001). Improving pineapple micropropagation protocol through explant size and medium composition manipulation. *Fruits, 56*(3), 143-154.

Daquinta, M., Cisneros, A., Rodriguez, Y., Escalona, M., Perez, M., Luna, I., & Borroto, C. (1997). Somatic embryogenesis in pineapple (Ananas comosus (L.) Merr). II International Pineapple Symposium 425,

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics, 11*(1), 1-42.

Escalona, M., Lorenzo, J., González, B., Daquinta, M., González, J., Desjardins, Y., & Borroto, C. (1999). Pineapple (Ananas comosus L. Merr) micropropagation in temporary immersion systems. *Plant cell reports, 18*(9), 743-748.

Fernando, K. (1986). In Vitro propagation of mauritius pineapple. *Tropical Agriculturist (Sri Lanka)*.

Firoozabady, E., & Gutterson, N. (2003). Cost-effective in vitro propagation methods for pineapple. *Plant cell reports, 21*(9), 844-850.

Hamad, A., & Taha, R. (2003). The effect of hormones on tissue culture of pineapple. *Jur Sains, 11*(1), 32-37.

Hamad, A., & Taha, R. (2009). Effect of explants density on the in vitro proliferation and growth of separated and cluster shoots of smooth cayenne pineapple (Ananas comosus L. Merr.). *Asian Journal of Plant Sciences, 8*(4), 313.

Hamad, A. M., & Taha, R. (2008). Effectof benzylaminopurine (BAP) on in vitro proliferation and growth of pineapple (Ananas Comosus L. Merr.) cv. Smooth cayenne. *Journal of Applied Sciences, 8*(22), 4180-4185.

Hamad, A. M., & Taha, R. M. (2008a). The effect of different hormones and incubation periods on in vitro proliferation of pineapple (Ananas comosus L.) Merr cv. Smooth Cayenne) shoot-tip culture. *Pak. J. Biol. Sci, 11*(3), 386-391.

Hamad, A. M., & Taha, R. M. (2008b). Effect of sequential subcultures on in vitro proliferation capacity and shoot formations pattern of pineapple (Ananas comosus L. Merr.) over different incubation periods. *Scientia Horticulturae, 117*(4), 329-334.

Khan, S., Nasib, A., & Saeed, B. A. (2004). Employment of in vitro technology for large scale multiplication of pineapples (Ananas comosos). *Pakistan Journal of Botany, 36*(3), 611-616.

Kofi, O., & Adachi, T. (1993). Effect of cytokinins on the proliferation of multiple shoots of pineapple in vitro. *SABRAO Journal, 25*(1), 59-69.

Konan, E. K., Kouadio, J. Y., Flori, A., Durand-Gasselin, T., & Rival, A. (2007). Evidence for an interaction effect during in vitro rooting of oil palm (Elaeis guineensis Jacq.) somatic embryo-derived plantlets. *In Vitro Cellular & Developmental Biology-Plant, 43*(5), 456-466.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays

ISSN: online 2617-2186 print 2617-2178

with tobacco tissue cultures. *Physiologia plantarum, 15*(3), 473-497.

Nelson, B. J., Asare, P. A., & Junior, R. A. (2015). In vitro Growth and Multiplication of Pineapple under Different Duration of Sterilization and Different Concentrations of Benzylaminopurine and Sucrose. *Biotechnology, 14*(1), 35.

Pérez, G., Yanes, E., Isidrón, M., & Lorenzo, J. C. (2009). Phenotypic and AFLP characterization of two new pineapple somaclones derived from in vitro culture. *Plant Cell, Tissue and Organ Culture, 96*(1), 113-116.

Pérez, G., Yanez, E., Mbogholi, A., Valle, B., Sagarra, F., Yabor, L., Aragón, C., González, J., Isidrón, M., & Lorenzo, J. C. (2012). New pineapple somaclonal variants: P3R5 and Dwarf. *Am J Plant Sci, 3*, 1-11.

Soneji, J. R., Rao, P., & Mhatre, M. (2002). Somaclonal variation in micropropagated dormant axillary buds of pineapple (Ananas comosus L., Merr.). *The Journal of Horticultural Science and Biotechnology, 77*(1), 28-32.

Sripaoraya, S., Marchant, R., Brian Power, J., & Davey, M. R. (2003). Plant regeneration by somatic embryogenesis and organogenesis in commercial pineapple (Ananas comosus L.). *In Vitro Cellular and Developmental Biology-Plant, 39*(5), 450-454.

Sunitibala Devi, Y., Mujib, A., & Kundu, S. (1997). Efficient regenerative potential from long term culture of pineapple. *Phytomorphology, 47*(3), 255-259.

Teixeira, S. L., Ribeiro, J. M., & Teixeira, M. T. (2006). Influence of NaClO on nutrient medium sterilization and on pineapple (Ananas comosus cv Smooth cayenne) behavior. *Plant Cell, Tissue and Organ Culture, 86*(3), 375-378.

Zuraida, A., Shahnadz, A. N., Harteeni, A., Roowi, S., Radziah, C. C., & Sreeramanan, S. (2011). A novel approach for rapid micropropagation of maspine pineapple (Ananas comosus L.) shoots using liquid shake culture system. *African Journal of Biotechnology, 10*(19), 3859-3866.

ISSN: online 2617-2186 print 2617-2178

تأثير فترة التحضين، حجم الوسط وكثافة العزلة على تكوين ونمو الفريعات وتكلفة انتاج مرحلة التضاعف في مزرعة أنسجة انااناس صنف موريس

عبد الحميد مختار حمد

قسم البستنة، كلية الزراعة، جامعة عمر المختار البيضاء– ليبيا

تاريخ الاستلام: 9 فبراير 2017 / تاريخ القبول: 20 ابريل 2017
https://doi.org/10.54172/mjsc.v33i2.290:Doi

المستخلص : أجريت هذه الدراسة لمعرفة درجة تأثير توليفات مكونة من أربعة فترات تحضين (30 ، 45 ، 60 و75 يوم) ثلاث درجات كثافة من العزلة (واحد ، اثنين و ثلاثة) وأربع كميات من الوسط (3 ، 6 ، 9 و 12 مل) علي عدد الفريعات المتكونة من العزلة الواحدة من أناناس صنف موريس وكذلك تأثيرها علي العدد الاجمالي للفريعات. التكلفة الاجمالية وتكلفة إنتاج الفريع الواحد فيما لو استخدم لتر واحد من الوسط. توليفة من ثلاث عزلات في 12 مل من الوسط احتضنت لمدة 60 يوم، ثلاث عزلات في 9 وفي 12 مل من الوسط وعزلة واحدة في 3 وفي 9 مل من الوسط احتضن كل منها لمدة 75 يوم ادت للحصول علي أعلي عدد من الفريعات المتكونة من العزلة الواحدة (11 فريع) ولكن اختلفت في العدد الاجمالي من الفريعات (2750 ، 3667، 2750، 3667 و 1222 فريع) وفي اجمالي التكلفة (25.85، 95.32، 28.39، 36.09 و 36.09 دولار امريكي علي التوالي). التوليفات التي استخدمت فيها ثلاث عزلات في المزرعة الواحدة أدت إلى اقل تكلفة انتاج للفريع الواحد (1.0 سنت) بينما التوليفات التي استخدم فيها عزلة واحدة تضاعفت تكلفة انتاج الفريع الواحد مرتين إلى ثلاث مرات (2.6 و 2.9 سنت). من ناحية أخري توليفة من ثلاث عزلات ، 3 مل من الوسط و 75 يوم تحضين أدت إلى الحصول علي 6 فريعات فقط (نصف أعلي متوسط فريعات امكن الحصول عليه) ولكن على أعلى اجمالي عدد من الفريعات (6000 فريع) وبأعلى اجمالي تكلفة (95.32 دولار) فيما لو استخدم لتر من الوسط وبتكلفة 1.6 سنت للفريع الواحد. تحضين ثلاث عزلات في 6 مل من الوسط لمدة 75 يوم أدي للحصول علي 10 فريعات من العزلة الواحدة وانتاج 5000 فريع وإجمالي تكلفة 50.68 دولار فيما لو استخدم لتر من الوسط وبتكلفة 1.0 سنت للفريع الواحد. استخدام عزلة واحدة و 12 مل من الوسط والتحضين لمدة 30 يوم أدى إلى الحصول علي 5 فريعات من العزلة الواحدة وبأعلى تكلفة إنتاج للفريع الواحد (5.0 سنت) ولكن أدني اجمالي عدد فريعات (417 فريع) وأقل اجمالي تكلفة (20.73 دولار) فيما لو استخدم لتر واحد من الوسط .

الكلمات المفتاحية : إجمالي الفريعات، التكلفة الإجمالية، حجم الوسط، كثافة العزلة، فترات التحضين، أناناس.

*عبد الحميد مختار حمد : abdelhamidhamad@gmail.com ، كلية الزراعة، جامعة عمر المختار البيضاء– ليبيا.

Al-Mukhtar Journal of Sciences 33 (2): 90-97, 2018

Risk factors of Atopic dermatitis in 1- to 4-Years Old Children in Al-Bayda city, Libya

Marfoua. S. Ali*[1], Samia M. Efkeren[1], Salema R.M Qowaider[2] and Salma. A. Bianco[3]

[1]*Zoology Department, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda, Libya*
[2]*Microbiology and Immunology Department, Faculty of Medicine,, Omar Al-Mukhtar University, Al-Bayda, Libya*
[3]*Razi Med Lab- El-Bayda, Libya*

Received: 31 July 2017/ Accepted: 11 November 2017
Doi: https://doi.org/10.54172/mjsc.v33i2.64

Abstract: Atopic dermatitis (AD) is common among pre-school children worldwide. Food allergy may be an important factor in children with atopic eczema under 4 years. Our objective was to assess the extent and characteristics of confirmed and unconfirmed diagnoses of AD in 112 children who were diagnosed by Pediatric Allergy Clinic at Al-Bayda's hospital with a high possibility of having AD at some point in their lifetimes. Sera from the patients were analyzed for specific IgE antibodies to 20 allergens. About 95% of children with AD have IgE-mediated clinical reactivity. The prevalence of positive cases with food allergens ranged from 19-20%, and environmental factors ranged from 11-14% for boys and girls respectively. Positive cases of environmental factors in this population were recorded against some species of plants. As a result, a combined sensitization to food and environmental allergens not only has an additive increase in serum IgE antibody production, but also increases the risk of developing allergic diseases such as asthma during childhood. An evaluation for AD should be considered to these children.

Keywords: Atopic dermatitis (AD), Food allergy, environmental factors, children, Al-Bayda and Libya

INTRODUCTION

Atopic dermatitis (AD) is the most common inflammatory skin disease in children, particularly in young children. The prevalence of AD has tripled over the past three decades and affects 15% to 30% of children in industrialized countries(Larsen, 1996) (Bieber, 2010). This growth has been too speedy to be explained by genetic changes and alteration environmental elements have been proposed as the possible explanation for the increased prevalence of AD(McNally et al., 1998). There are also many factors demonstrated to be associated with AD: personal factors including, age, gender, nutritional status, lifestyle, allergy status and family history (McNally et al., 1998) and changes in environmental factors (e.g. house dust mite, animal dander, molds, cockroach infestation, occupational exposure, environmental tobacco smoke, air pollution, aeroallergens, and climate change) (Lee et al., 2012; McNally et al., 2001; Schäfer et al., 2008). Since the first documented report of food allergy-provoking AD in 1915 (Schloss, 1915), parents and patients with AD continue to implicate food in disease flares (Greenhawt, 2010), an idea backed by the fact that food allergy is more common in children with AD. 30% of them are affected compared with 4% to 10% in the general pediatric population (Suh, 2010). Roughly one-third of kids with severe AD suffer from food allergy. In childhood, food

***Corresponding Author:** [1] Marfoua. S. Ali, marfouas@yahoo.com Faculty of Science, Omar El-Mukhtar University, El-Beyda, Libya

allergies and food allergens such as cow's milk or hen's egg are mainly responsible for allergic reactions, when compared with adolescents and adults (Wassmann & Werfel, 2015). The diagnosis of eczematous reactions to food demands a careful diagnostic procedure, taking into account the patient's history and sensitization patterns. Despite the large types of food that can cause IgE- mediated reactions, most prevalence studies have concentrated on the most common allergenic foods, namely cow's milk, hen's egg, peanut, tree nut, wheat, soya, fish, and shellfish. Such allergens account for up to 90% of food allergy reactions (Eigenmann et al., 1998). Various diagnostic tools for atopic dermatitis (AD) have been proposed due to the lack of definitive biomarkers and the marked diversity of its clinical features (Brenninkmeijer et al., 2008; Deleuran & Vestergaard, 2014). The aim of our study was to screen serum of a group of children with AD to determine the course of sensitization and the development of clinical allergy which include a common allergic food and some environmental factors.

MATERIALS AND METHODS

The study protocol was reviewed and approved by Bioethics Committee at Biotechnology Research Center (BEC-BTRC) with Ref No: BEC-BTRC 04-2017. A group of 112 children (age: 6 months -48 months) with atopic eczema (inflammatory skin disease characterized by an itchy red rash) diagnosed by a doctor in Pediatric Allergy Clinic and Outpatients department at Al-Bayda's Hospital, Libya. The physicians involved in the study protocol determined the diagnosis of AD as a high possibility to have food allergies. Questions included demographic information, age of onset of allergy, number of accidental exposures, and history of concurrent food allergies. Then, a specific kit was used to determine main causes of these symptoms. Serum samples from all patients were collected at the time of the visit in Razi Med Lab in Al-Bayda and analyzed for allergen–specific IgE antibodies using the ImmunoCAP Specific IgE (TÜV Rheinland, Germany) (Maloney et al., 2008) . In brief, venous blood samples were collected and analyzed with the automated ImmunoCAP System. Fifty microliters of standards or patients' sera were added to the solid matrix (ImmunoCAP) and incubated for 30 minutes at room temperature. After washing, enzyme-labeled anti-IgE was added to the ImmunoCAP and incubated for 2.5 hours at same temperature, after which it was washed again. Then, 50 mL of developing solution was added to each sample. After a 10-minute of incubation, a stopping solution was added and fluorescence was determined and compared with values from the standard curve using biocheck imaging software. With the help of Biocheck Imaging Software (BIS) and a PC, the Polycheck cassettes were interpreted (TÜV Rheinland, Germany). In comparison with the standard curve, the amount of allergen-specific IgE for each allergen was given as relative kilo units per liter (kU/l) and results were determined based on their concentration of IgE. In this test, we used a pediatric screen for 20 individual allergens for children, designed as a screening test for the most relevant inhalation and food allergens. Histamine (10 mg/ml) was used as a positive control and glycerine as a negative control. Allergen-specific IgE (sIgE) concentrations in the serum were determined for the following allergens: milk, casein, α-laktalbumin, β-laktoglobulin, Bovine serum albumin, egg yolk and egg white, rice, soybean, banana, pork, beef, chicken, flour mix, bakers-yeast, *D.pteronyssinus- D.farinaemix*, Cladosp herb.-Altern.altern.mix, Birch-Oak pollen mix, Alder-Hazel pollen mix, six Grass mix and level of IgE in serum. Degree of reaction for each allergen separately was ranged from 0.15 as a negative result (no specific antibody detection) to 100 as a positive result (very strong antibody concentration) that obtained as a result of collaboration of soft program with Polycheck kit.

ISSN: online 2617-2186 print 2617-2178

RESULTS

From January to December 2016, 112 cases were screened for specific serum IgE for 20 different allergens. The majority of children were having atopic dermatitis or asthma at some point in their lifetimes. Of the children with reported symptoms of AD, the patients ranged in age from 6 months to 4 years (median, 2.5 years), and the ratio of gender of patients was male/female ratio of 47:65. There was similarity of results found between couple of genders with a type of allergens and most of them have more than one positive reaction to different allergens. Results in Table 1 illustrated a comparison in numbers between percentages of negative and positive cases for food/environmental allergens in both genders. The prevalence of positive cases with food allergens ranged from 19-20%, and prevalence of environmental factors ranged from 11-14% for boys and girls respectively. Most cases got a positive reaction against more than one type of allergens, thus the total numbers for positive and negative cases of food allergens for boys were 252 and 723 respectively, whereas the total number of the boys and girls in this study were 47 and 65 respectively (Figure 1 showed an example of the results for one subject).

For more details, Table 2 presented the numbers with percentages of negative and positive cases, and the mean degrees of reaction for each gender. Overall boy/girl cases, the highest numbers of positive cases were found with Alder-Hazel pollen mix (38 boys/44 girls) and Birch-Oak pollen mix (31 boys/43 girls), and the lowest numbers were noted with α-Laktalbumin (2 boys/4 girls), Casein (3 boys) and Chicken (4 girls). Additionally, of these 47 boys, 27 had reacted to *D. pteronyssinus-,D. farinaemix*, banana, and 24 reacted to 6-Grass mix. Of these 65 girls, 36 had reacted to *D. pteronyssinus-D. farinaemix*, 39 reacted to a banana, and 21 reacted to 6-Grass mix. The results also showed that the total numbers for positive and negative cases of food allergens for boys were 252 and 723 respectively, whereas the total number of the boys/girls in this study was 47/56 subjects. This referred to the most case that had been found to be positive to more than one type of food/ environmental allergens at the same time. In the same table, the ranges of increased serum specific IgE levels for each allergen varied widely among patients with positive diagnoses, and the total mean level of IgE was 96 and 100% for boys and girls respectively. The same table showed levels of mean degree of reaction for each allergen that ranges between 0.15-100 kU/I (concentration of antibody detection). The highest value was recorded with *D. pteronyssinus-D.farinaemix* with 21.4 and 19.8 for boys and girls respectively, followed by 6-grass mix and banana.

Table (1). Numbers and percentages of negative and positive cases for food/environmental allergens in both genders

Parameters	Boys (47)		Girls (65)	
	cases tive posi-	cas- tive nega-	cases tive posi-	cases tive nega-
Total cases of food allergens	252 (19.38)	723 (55.62)	194 (20.64)	511 (54.36)
Total cases of environmental allergens	149 (11.46)	176 (13.54)	131 (13.94)	104 (11.06)

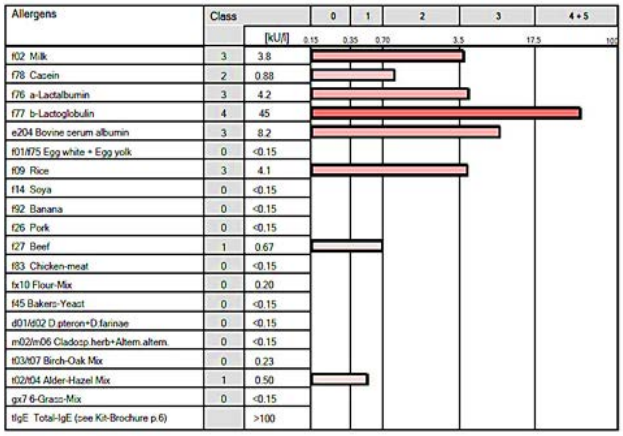


Figure (1). Results of Atopic test for one subject shown positve reaction for more than one type of allargens

ISSN: online 2617-2186 print 2617-2178

Table (2) .Correlation between different twenty allergens with percentages of positive/negative cases and mean degrees of reaction in male and female subjects

No	Atopic	Boys (47)			Girls (65)		
		No of negative cases (%)	No of positive cases (%)	Mean degree of reaction	No of negative cases (%)	No of Positive cases (%)	Mean degree of reaction
1	Milk	41 (87.234)	6 (12.766)	0.197	57 (87.962)	8 (12.308)	0.282
2	Casein	44 (93.617)	3 (6.383)	0.169	57 (87.962)	8 (12.308)	0.181
3	α-Laktalbumin	45 (95.745)	2 (4.255)	0.181	61 (93.846)	4 (6.154)	0.279
4	β-Laktoglobulin	30 (63.830)	17 (36.170)	0.648	33 (50.769)	32 (49.231)	2.085
5	Bovine serum albumin	34 (72.340)	13 (27.660)	1.711	46 (70.769)	19 (29.231)	0.627
6	Egg white + Egg yolk	39 (82.979)	8 (17.021)	0.177	54 (83.077)	11 (16.923)	0.189
7	Rice	32 (68.085)	15 (31.915)	0.580	47 (72.308)	18 (27.692)	1.117
8	Soybean	38 (80.851)	9 (19.149)	0.625	56 (86.154)	9 (13.846)	0.593
9	Banana	20 (42.553)	27 (57.447)	3.627	26 (40)	39 (60)	2.808
10	Pork	38 (80.851)	9 (19.149)	1.064	51 (78.462)	14 (21.538)	0.535
11	Beef	35 (74.468)	12 (25.532)	0.207	57 (87.962)	8 (12.308)	0.18
12	Chicken	40 (85.106)	7 (14.894)	0.203	61 (93.846)	4 (6.154)	0.162
13	Flour-Mix	31 (65.957)	16 (34.043)	0.420	48 (73.846)	17 (26.154)	0.475
14	Bakers-Yeast	24 (51.064)	23 (48.936)	1.266	40 (61.538)	25 (38.462)	0.319
15	*D.pteronyssinus*-*D.farinaemix*	20 (42.553)	27 (57.447)	21.355	29 (44.615)	36 (55.385)	19.814
16	Cladosp.herb.-Altern.altern.mix	36 (76.596)	11 (23.404)	0.302	60 (92.308)	5 (7.692)	0.336
17	Birch-Oak pollen mix	16 (34.043)	31 (65.957)	0.575	22 (33.846)	43 (66.154)	1.086
18	Alder-Hazel pollen mix	9 (19.149)	38 (80.851)	0.420	21 (32.308)	44 (67.692)	0.916
19	6-Grass mix	23 (48.936	24 (51.064)	3.980	44 (67.692)	21 (32.308)	2.886
20	Total IgE	2 (4.255)	45 (95.745)	58.42	0	65 (100)	59.351

DISCUSSION

AD/food allergy use had distinct risk factors. The prevalence of sensitization to food allergens appeared to occur and increase in early infancy (Kulig et al., 1998). In young children, the diagnosis of AD is mainly based on clinical evaluation. Although assessment of allergen-specific IgE antibodies provides helpful information to the clinician, a correct interpretation of sensitization to common allergens is critical in determining susceptibility to allergic diseases (Chiu et al., 2014). This study attempted to estimate prevalence and risk factor profile of AD/food allergy that cause allergy in early childhood by comparing the distribution of different allergens in subjects suffers from AD. From our result, close to 95% of children with AD have IgE-mediated clinical reactivity. As a result, an

ISSN: online 2617-2186 print 2617-2178

allergic reaction occurs when the immune system overreacts to the allergen by producing IgE antibodies. IgE is a type of antibody that is presented in small amounts in the body but plays a major role in allergic diseases (Galli & Tsai, 2012). In this study, the changes of serum IgE levels were consistent with the sensitization patterns of food. The lowest numbers of positive cases noted with α-Laktalbumin (2 boys/4 girls), Casein (3 boys) and Chicken (4 girls). On the other hand, the highest number of positive cases was recorded with banana followed by Bakers-Yeast, then β-Laktoglobulin, flour-mix, and rice for both genders. These results might refer to the fact that these types of food are considered as an important food for children at an early age. The total positive case with food allergens was found around 20% for both genders. These results were in agreement with other studies that found food allergy as an important factor in up to 20% of children with atopic eczema under 4 years (Oranje & De Waard-Van Der Spek, 2000; Tariq et al., 2000). These results were close to the finding of another study which stated that children (1 to 10 years old) in German were found to have a positive predictive value for eczematous reactions with food of 30% (Breuer et al., 2004). In another study, only 35% to 40% of food-sensitized children with AD have clinical signs and symptoms of food allergy (Greenhawt, 2010).

On the other hand, from our results, percentages of total positive cases with environmental allergens were found to be around 11-14% in both genders. In more details, 80 and 77% of subjects were found to have a high positive reaction with Alder-Hazel pollen mix, followed by Birch-Oak pollen mix and *D.pteronyssinus -D.farinaemix* compared to food allergens. This might be attributed to the presence of these variety of plants in study area. Our results agreed with a study that found a prevalence of AD of (11.7%) from pediatric dermatosis in Benghazi, Libya (Elfaituri, 2015). They also agreed with a study that found approximately half of young infants with food allergen sensitization were more likely to be allergic to inhalant allergens, and were at an increased risk of developing respiratory allergic diseases by the age of 4 years (Chiu et al., 2014). Thses environmental factors might play a role in increasing the risk of asthma which was reported in another study with children at age 1-14years in the same city. Results of that study found a high number of children with severe smoke sensitivity and dust allergy and that the environmental factors might lead to the development of asthma in older children (Ali *et al.* 2016). To sum up, not only lifestyles but also environmental factors may be associated with the prevalence of AD in children at an early age in Al-Bayda city, which might lead to the increased risk of developing a respiratory allergy. This risk is high especially in children with early onset of food allergy.

CONCLUSION

The complex interplay between the variability of the environmental exposure and the interactions between the food allergies are likely to affect the development of AD. It is important to identify infants at risk to provide early intervention. Prevention should begin in early life, a critical window of vulnerability. Fixing of the epidermal barrier in infants with AD may prevent the subsequent allergic diseases.

Consent

It is not applicable.

ACKNOWLEDGMENTS

The authors would like to acknowledge the workers in Razi Med Lab and patients in Al-Bayda city.

COMPETING INTEREST

Authors have declared that no competing interests exist.

ISSN: online 2617-2186 print 2617-2178

REFERENCES

Ali, M.S. Mekal, F.H. Taib, R. M. and Qowaider, S. R. (2016). Prevalence and risk factors of childhood Asthma in Al-Bayda city in Northeast of Libya. Journal of Pharmacy and Biological Sciences 11 (5): 115-118.

Bieber, T. (2010). ATOPIC dermatitis. Annals of Dermatology 22(2): 125-137.

Brenninkmeijer, E., Schram, M., Leeflang, M., Bos, J., & Spuls, P. I. (2008). Diagnostic criteria for atopic dermatitis: a systematic review. *British Journal of Dermatology, 158*(4), 754-765.

Breuer, K., Heratizadeh, A., Wulf, A., Baumann, U., Constien, A., Tetau, D., Kapp, A., & Werfel, T. (2004). Late eczematous reactions to food in children with atopic dermatitis. *Clinical & Experimental Allergy, 34*(5), 817-824.

Chiu, C.-Y., Huang, Y.-L., Tsai, M.-H., Tu, Y.-L., Hua, M.-C., Yao, T.-C., Yeh, K.-W., & Huang, J.-L. (2014). Sensitization to food and inhalant allergens in relation to atopic diseases in early childhood: a birth cohort study. *PloS one, 9*(7), e102809.

Deleuran, M., & Vestergaard, C. (2014). Clinical heterogeneity and differential diagnosis of atopic dermatitis. *British Journal of Dermatology, 170*(s1), 2-6.

Eigenmann, P. A., Sicherer, S. H., Borkowski, T. A., Cohen, B. A., & Sampson, H. A. (1998). Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics, 101*(3), e8-e8.

Elfaituri, S. S. (2015). Pediatric dermatoses in Benghazi, Libya. *Indian Journal of Paediatric Dermatology, 16*(2), 64.

Galli, S. J., & Tsai, M. (2012). IgE and mast cells in allergic disease. *Nature medicine, 18*(5), 693-704.

Greenhawt, M. (2010). The role of food allergy in atopic dermatitis. Allergy and asthma proceedings,

Kulig, M., Bergmann, R., Tacke, U., Wahn, U., & Guggenmoos‐Holzmann, I. (1998). Long‐lasting sensitization to food during the first two years precedes allergic airway disease. *Pediatric allergy and immunology, 9*(2), 61-67.

Larsen, F. S. (1996). Atopic dermatitis: an increasing problem. *Pediatric allergy and immunology, 7*(S9), 51-53.

Lee, J. H., Suh, J., Kim, E. H., Cho, J. B., Park, H. Y., Kim, J., Ahn, K., Cheong, H. K., & Lee, S.-I. (2012). Surveillance of home environment in children with atopic dermatitis: a questionnaire survey. *Asia Pacific Allergy, 2*(1), 59.

Maloney, J. M., Rudengren, M., Ahlstedt, S., Bock, S., & Sampson, H. A. (2008). The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. *Journal of Allergy and Clinical Immunology, 122*(1), 145-151.

McNally, N., Williams, H., & Phillips, D. R. (2001). Atopic eczema and the home environment. *British Journal of Dermatology, 145*(5), 730-736.

McNally, N. J., Phillips, D. R., & Williams, H. C. (1998). The problem of atopic eczema: aetiological clues from the environment and lifestyles. *Social Science & Medicine, 46*(6), 729-741.

Oranje, A., & De Waard-Van Der Spek, F. (2000). Atopic dermatitis and diet. *Journal of the European Academy of*

ISSN: online 2617-2186 print 2617-2178

Dermatology and Venereology, 14(6), 437-438.

Schäfer, T., Stieger, B., Polzius, R., & Krauspe, A. (2008). Atopic eczema and indoor climate: results from the children from Lübeck allergy and environment study (KLAUS). *Allergy, 63*(2), 244-246.

Suh, K.-Y. (2010). Food allergy and atopic dermatitis: separating fact from fiction. Seminars in cutaneous medicine and surgery,

Tariq, S. M., Matthews, S. M., Hakim, E. A., & Arshad, S. H. (2000). Egg allergy in infancy predicts respiratory allergic disease by 4 years of age. *Pediatric allergy and immunology, 11*(3), 162-167.

Wassmann, A., & Werfel, T. (2015). Atopic eczema and food allergy. In *Food Allergy: Molecular Basis and Clinical Practice* (Vol. 101, pp. 181-190). Karger Publishers.

ISSN: online 2617-2186 print 2617-2178

التهاب الجلد التأتبي(Atopic Dermatitis) وبعض العوامل المسببة له في الأطفال من سن 1-4 سنوات في مدينة البيضاء-ليبيا

مرفوعة صالح علي [1] . سامية محمد افكيرين [1] ، سليمة رزق الله اقويدر [2] وسالمة عبدالحميد بيانكو [3]

[1] قسم علم الحيوان، كلية العلوم، جامعة عمر المختار، البيضاء-ليبيا

[2]قسم علم الأحياء الدقيقة والمناعة، كلية الطب البشري، جامعة عمر المختار، البيضاء – ليبيا

[3] معمل الرازي للتحاليل- البيضاء- ليبيا

تاريخ الاستلام: 31 يوليو 2017 / تاريخ القبول: 11 نوفمبر 2017

Doi:https://doi.org/10.54172/mjsc.v33i2.64

المستخلص : التهاب الجلد التأتبي (Atopic Dermatitis) هو أمر شائع بين الأطفال في مرحلة ما قبل سن الدراسة في جميع أنحاء العالم، والمسبب لالتهاب الجلد التأتبي ليس معروفًا. قد تكون الحساسية الغذائية من أهم العوامل المؤثرة والمسببة في معاناة الأطفال من هذا المرض أعمارهم من سنة واحدة إلى أربع سنوات. هدف هذه الدراسة هو تقييم مدى تشخيص المرض في عدد 122 حالة بعضها تم تأكيد التشخيص بها وأخرى مشكوك في تشخيصها حيث أن احتمال الإصابة تم تشخيصه في مرحلة ما من حياتهم من قبل عيادة حساسية الأطفال في مستشفى البيضاء. تم تحليل السيرم عند المرضى لتقدير نوع ومستوى الأجسام المضادة محددة لعدد 20 مسبباً من مسببات الحساسية، ما بين مسببات غذائية وأخرى بيئية. أظهرت النتائج أن حوالي 95٪ من الأطفال المصابين بالتهاب الجلد التأتبي لديهم تفاعل إيجابي للجسم المضاد IgE، وقد تراوحت نسبة الحالات الإيجابية مع المواد المسببة للحساسية الغذائية بين 19-20٪، والحالات الإيجابية للعوامل البيئية بين 11-14٪ للذكور والإناث على التوالي، وقد كانت الحالات إيجابية لعوامل الحساسية الغذائية في فئة الدراسة ضد بعض أنواع النباتات. الخلاصة التي توصل لها البحاث هو التوعية المجتمع بالمكونات المسببة للحساسية في المواد الغذائية، فأي زيادة في إنتاج الأجسام المضادة IgE في المصل يمكن أن يزيد من خطر الإصابة بأمراض الحساسية مثل الربو في مرحلة الطفولة.

الكلمات المفتاحية : التهاب الجلد التأتبي، الحساسية الغذائية، الأطفال تحت سن 4 سنوات، البيضاء، ليبيا.

*مرفوعة صالح علي :marfouas@yahoo.com ،كلية العلوم، جامعة عمر المختار، البيضاء- ليبيا.

Al-Mukhtar Journal of Sciences 33 (2): 98-103, 2018

Prevalence of Irritable Bowel Syndrome (IBS) in Lamar- Libya Primary Care General practices

Salem Awami*, Faraj Alhomry and Najib Mohamed

Department of medicine, Faculty of medicine, Omar AL-Mukhtar University, El-Bayda, Libya

Received: 15 October 2017 / Accepted: 11 December 2017
Doi: https://doi.org/10.54172/mjsc.v33i2.113

Abstract: Irritable bowel syndrome (IBS) is a chronic or recurrent abdominal symptom. No cause can be identified using conventional diagnostic testing and it is characterized by abdominal pain or cramping and changes in bowel function. Aims of our study were to estimate the prevalence of functional bowel disorders namely C1; Irritable bowel syndrome (IBS) among clinic-based patients, and to assess health-care seeking in subjects with functional bowel disorders. The study used participants recruited from AL Marj- Libyan Red Crescent Clinic primary care general practices. Between November 2012 and December 2013, each study subject was asked according to a prepared questionnaire. This questionnaire depends on Rome III diagnostic criteria for irritable bowel syndrome. 450 consecutive patients attended the Red Crescent Clinic, 234 (52%) were female and 216 (48 %) were male. Population age ranged from 20-80 years, and the mean age was 53 years (SD+ 15.6). In our study, the prevalence of irritable bowel syndrome (IBS) is 12%, and 53.3% of the subjects had looked for medical advice for abdominal pain. There was no significant association observed between the prevalence of IBS and sex. IBS was11.9% in women and 12.1% in men. Elderly were the least frequent sufferer of IBS and the least medical advice seeker compared to younger age groups, which necessitate not overlooking organic cause in such age group. Considerable patients seek medication and traditional remedies due to their bowel disorders. Further investigations of the treatment are required because of the high financial burden for individuals as well as for the society.

Keywords: Irritable bowel syndrome, IBS, Rome III diagnostic criteria, abdominal pain or discomfort, Functional gastrointestinal disorders, (FGID).

INTRODUCTION

Functional gastrointestinal disorders (FGID) are clinical syndromes defined by chronic or recurrent abdominal symptoms. No cause can be identified using conventional diagnostic testing. They can be classified by anatomic region: esophageal (A), gastroduodenal (B), bowel (C), functional abdominal pain (D), biliary (E), and anorectal (F). Within each anatomic category site, there can be several disorders each with specific clinical features. For example, the functional bowel disorders (C), which include IBS (C1), functional abdominal bloating (C2), functional constipation (C3), and functional diarrhea (C4), are all functional bowel disorders attributed to the colon and rectum (Table 1) (Drossman, 2006). Irritable bowel syndrome (IBS) is a complex symptom characterized by abdominal discomfort or pain associated with defecation or a change in bowel habit. IBS is a common disorder with a prevalence of 15–20% in the general population and it constitutes 50% of the cases in outpatient clinics of gastroenterology. It appears that 33–90% of sufferers do not consult their clinicians, and that a proportional meeting IBS criterion is not labeled as having IBS by their clinicians. (Talley *et al.* 1992b, Mertz, 2003). IBS is not associated with the development of long-term serious disease and there is no evidence to

***Corresponding Author:** Salem Awami, salemawami@yahoo.com Faculty of Medcine, Omar EL-Moukhtar University, El-Beida, Libya

link IBS to excess mortality, although it has been shown that patients with IBS are more likely to undergo certain surgical operations including hysterectomy and cholecystectomy compared to matched non-IBS controls. Health-related quality of life is poor in patients with IBS and can lead to a loss of time for work or increased health care costs (Spiller *et al.* 2007).Symptoms of Functional bowel disorder are abdominal pain or discomfort which is clearly linked to bowel function, being either relieved by defecation (suggesting a colonic origin) or associated with a change in stool frequency or consistency suggesting a link to changes in intestinal transit, which might reflect changes in either motor patterns or secretion. (Horwitz & Fisher, 2001).

Table 1: Functional bowel disorders; bowel disorders category C (Talley et al. 1999)

C1. Irritable bowel syndrome
C2. Functional abdominal bloating
C3. Functional constipation
C4. Functional diarrhea

Aims of our study were to estimate the prevalence of functional bowel disorders C1; IBS among clinic-based patients, and to assess health-care seeking in subjects with functional bowel disorders.

MATERIALS AND METHODS

The study used participants recruited from AL Marj- Libyan Red Crescent Clinic general practices. For each study subject, date of birth and sex were registered by a doctor who also asked every study subjects according to a prepared questionnaire. This questionnaire depends on Rome III diagnostic criteria for irritable bowel syndrome which was created by the Rome III committee, (Table 2), (Appendix A, 2006). The questionnaire assessed abdominal pain or discomfort in the last 12 months. Abdominal disorders when occurred, the number of episodes, and associated symptoms were assessed. These symptoms are common in IBS but not part of the diagnostic criteria namely; bloating, straining at defecation, urgency, feeling of incomplete evacuation, and the passage of mucus per rectum. Subjects were asked if pain or discomfort improved after defecation and if it was associated with altered stool habits (more or less bowel movements, harder or looser stools). History of follow up with a doctor or alternative medical care because of abdominal complaints was recorded.

Table 2: Rome III diagnostic criteria* for irritable bowel syndrome

Recurrent abdominal pain or discomfort at least 3 days a month in the past 3 months, associated with two or more of the following: • Improvement with defecation • Onset associated with a change in frequency of stool • Onset associated with a change in form (appearance) of stool
*Criteria fulfilled for the past 3 months with symptom onset at least 6 months before diagnosis

Translation and cultural adaptation

The questionnaire was translated into Arabic according to the international principles (WHO, 2013). As a part of the translation process, the Arabic language versions were tested with IBS patients. The questionnaire was back translated into English after translation into Arabic and reviewed again by members of the questionnaire survey team to ensure preservation of content and clarity of the items (Gorecki, *et al.* 2013).

RESULTS

Between November 2012 and December 2013, 450 consecutive patients attended the AL Marj- Libyan Red Crescent primary care clinic, 234 (52%) were female and 216 (48 %) were male, population aged 20-80 years, and mean age was 53 years (SD+ 15.6), with 38%, 35%, and 27% in the age groups 20-40, 41-60, and 61-80 years respectively. There

ISSN: online 2617-2186 print 2617-2178

were 240 subjects (53.3%) reported abdominal discomfort or pain at least once in the previous 12 months. 130 (29%) subjects had discomfort occurred more than 6 times during the past year. 99 (22%) subjects reported lower abdominal pain, and 54 (12%) had IBS. Prevalence of seeking medical care due to lower abdominal pain was lower in subjects aged 60-80 years compared to subjects aged 20-40 years. The prevalence of IBS was significantly lower in the age groups 60-80 and 40-60 years compared to 20-40 years old subjects (Table 3). 256 subject (56%) had reported intake of drugs prescribed by their doctors and 170 (37%) reported intake of different remedies (traditional treatment or herbal treatment) for abdominal symptoms in the past 12 months.

Table (3): prevalence of IBS and abdominal pain, for sex and age per 100 population

	Abdominal pain %	IBS %
All subjects	22	12
Sex		
Male	21.8	12.1
Female	22.2	11.9
Age groups (years)		
20-40	26.6	17.5
40-60	22.6	11
60-80	16.8	9.6

DISCUSSION

Our study was not different from international reported studies on the prevalence of Irritable bowel syndrome (IBS) which is 12%. (Icks *et al.* 2000), 53.3% had looked for medical advice for abdominal pain. (Talley & Boyce 1979) had reported the results of a population- based study which attempted to explain health care seeking for irritable bowel syndrome (IBS) patients. Of their sample, 13% had IBS, and of this, 73% had sought medical care for abdominal pain or discomfort. (Ringström *et al.* 2007). There was no significant association observed between the prevalence of Irritable bowel syndrome (IBS) and sex in our study. IBS was in women 11.9% and in men 12.1%. We might attribute that to the clinic based questionnaire, although in the literature there was nothing to support differences in prevalence among different sexes because female subjects had more GI-related health-seeking behavior than the male counterpart, whereas no gender difference existed in terms of previous medical or intra-abdominal surgical history, education, school/ work absenteeism and sleep disturbance. (Lu *et al.* 2006; Chang *et al.* 2010). In our study we found that Functional bowel disorder in the elderly was less frequent compared to younger age groups, and the prevalence of seeking medical care due to abdominal pain was lower in subjects aged 60-80 years compared to subjects 20-40 years. This is very important to draw our attention to organic cause for GIT symptoms in the elderly along with other alarming symptoms (table 4). As in age over 50 years at onset of symptoms, male sex, blood mixed in the stool, and blood on the toilet paper were all predictors of an organic diagnosis. (Talley *et al.* 1992a).

Table (4): Alarm features in irritable bowel syndrome (Spiller et al. 2007)

- Age 50 years
- Short history of symptoms
- Documented weight loss
- Nocturnal symptoms
- Male sex
- Family history of colon cancer
- Anemia
- Rectal bleeding
- Recent antibiotic use

More than half of the patients with lower abdominal pain (56%) had mentioned that they had taken prescribed drugs from their doctors due to their abdominal symptoms in the past 12 months, and more than one third of them (37%) have used different remedies (traditional treatments or herbal treatments) (Ikechi et al., 2017), and probiotics and prebiotics, (Lawrence & Hyde, 2017). This indicates considerable patients seek medication and traditional remedies due to their bowel disorders. Further investigations of treatments are

ISSN: online 2617-2186 print 2617-2178

required because of the high financial burden for individuals as well as for the society.

CONCLUSION

Living with functional bowel disorder represents daily challenges. It may be painful or embarrassing and can seriously affect the quality of life. As yet there is no imaging test to aid in diagnosis, which relies upon history. Although many adults have signs and symptoms of functional bowel disorder, fewer than half seek medical help. Yet it's important to discover alarming symptoms especially after the age of 50 years.

ACKNOWLEDGEMENTS

We thank Libyan Red crescent clinic at Al marj city, the administration and medical staff for helping and allowing us to conduct the study and using their resources.

REFERENCES

Chang, F. Y. Lu, C. L. and Chen, T. S. (2010). The current prevalence of irritable bowel syndrome in Asia." Journal of neurogastroenterology and motility 16(4): 389-400.

Drossman, D.A. (2006). "Rome III: the new criteria." Journal of Digestive Diseases **7**(4): 181-185.

Gorecki, C. Brown J. Cano, S. Lamping, D. Briggs, M. Coleman, S. Dealey, C. McGinnis, E. Nelson, A, Stubbs, N. Wilson, L. and Nixon, J. (2013). "Development and validation of a new patient-reported outcome measure for patients with pressure ulcers: the PU-QOL instrument." Health and quality of life outcomes 11(1): 95.

Horwitz, B. J. and Fisher, R. S. (2001). "The irritable bowel syndrome." New England Journal of Medicine 344(24): 1846-1850.

Icks, A. Rathmann, W. Enck, P. Giani, G. (2000)." What is the real prevalence of the IrritableBowel Syndrome?" Gastroentero -logy; 118 (Suppl.): A717

Ikechi, R. Bradford, D. De Sipio, F. J. and Phadtare, S. (2017).Irritable Bowel Syndrome: Clinical Manifestations, Dietary Influences, and Management. Healthcare Multidisciplinary Digital Publishing Institute.

Lawrence, K. and Hyde, J. (2017). Microbiome restoration diet improves digestion, cognition and physical and emotional wellbeing . PloS one 12(6): e0179017.

Lu, C. L. Chang, F. Y. C. Chen, Y. , J. Luo, C. and Lee, S. D. (2006). Significance of Rome II-defined functional constipation in Taiwan and comparison with constipation-predominant irritable bowel syndrome. Aliment Pharmacol Ther 24:429–438.

Mertz, H. R. (2003). Irritable Bowel Syndrome. The New England Journal of Medicine; 349(22):2136-2146.

Ringström, G. Abrahamsson H. Strid H. and Simrén, M. (2007).Why do subjects with irritable bowel syndrome seek health care for their symptoms? Scandinavian Journal of Gastroenterology 42(10): 1194-1203.

Spiller, R. Aziz, Q. Creed, F. Emmanuel, A. Houghton, L. Hungin, P. Jones, R. Kumar, D. Rubin, G. Trudgill, N. and Whorwell, P. (2007).Guidelines on the irritable bowel syndrome: mechanisms and practical management. Gut. 56(12):1770–1798

Talley, N. J. Boyce, P. M. and Jones, M. (1997). Predictors of health care seeking for irritable bowel syndrome: a population based study. Gut. 41: 394–398

ISSN: online 2617-2186 print 2617-2178

Talley, N. J. O'Keefe, E. A. Zinsmeister, A. R. and Melton, L.J. (1992a).Prevalence of gastrointestinal symptoms in the elderly: A population-based study. Gastroentero –logy 102: 895-901.

Talley, N. J. Stanghellini, V. Heading, R. C. Koch, K. L. Malagelada, R. Tytgat, G. N. (1999).Functional gastroduodenal disorders. Gut. 45 (Suppl II):II37–II42

Talley, N.J. Weaver, A.L. Zinsmeister, A.R. and Melton, L.J. (1992b).Onset and disappearance of gastrointestinal symptoms and functional gastrointestinal disorders. American Journal of Epidemiology 136(2): 165-177.

World Health Organization. (2013). Process of translation and adaptation of instruments WHO.

ISSN: online 2617-2186 print 2617-2178

مدي شيوع متلازمة القولون العصبي لمترددي عيادات الهلال الاحمر للرعاية الأولية بمدينة المرج ليبيا

سالم العوامي*، فرج الحمري ونجيب صالح

قسم الامراض الباطنة كلية الطب البشري جامعة عمر المختار، البيضاء. ليبيا

تاريخ الاستلام: 15 اكتوبر 2017 / تاريخ القبول: 11 ديسمبر 2017.
Doi:https://doi.org/10.54172/mjsc.v33i2.113

المستخلص : متلازمة القولون العصبي هي إحدى اضطرابات الجهاز الهضمي المزمنة والمتكررة، وتتميز بآلام البطن المختلفة لكنها تثير الحيرة بسبب الاضطراب الوظيفي للقولون وعدم وجود مرض عضوي واضح أو تغيرات هامة في الفحوصات المخبرية، والأشعة السينية، وكذلك تصوير البطن والمناظير الطبية أو الفحص النسيجي. الغرض من هذه الدراسة تقدير مدي انتشار الامراض الوظيفية خصوصا أمراض القولون العصبي بين المرضي مترددي العيادات العامة وكذلك تقدير قبول وبحث الأشخاص على الخدمات الطبية والعلاج. تم اختيار مشاركين بهذه الدراسة من عيادات الرعاية الأولية للهلال الأحمر الليبي بمدينة المرج ليبيا من الفترة بين نوفمبر 2012 الي ديسمبر 2013، تم سؤال كل مشارك عن طريق استبيان يعتمد علي توصيات اجتماع روما الثالث الخاص بتشخيص القولون العصبي ، حضر 450 مشارك لعيادة الهلال الاحمر 234 (52%) نساء و216 (48 %) رجال، تراوحت اعمار المشاركين بين20 - 80 سنة، متوسط العمر 53 سنة (انحراف معياري ± 15.6). بينت هذه الدراسة شيوع القولون العصبي بنسبة 12% وإن 53.3% من المشاركين بحثوا عن المساعدة الطبية بسبب الام البطن. لا يوجد ارتباط بين شيوع القولون العصبي وجنس المريض وذلك لتعادل نسبة المرض بين الجنسين تقريبا (11.9%في النساء و12.1.% الرجال). وأوضحت هذه الدراسة عزوف المسنين عن المساعدة الطبية مقارنة بالأصغر سناً، وكذلك وجود عدد كبير من المشاركين يلجؤون للتداوي بالطب البديل. كما يحتاج هذا المرض لمزيد من الابحاث وذلك لشيوعه وقلة الدواء الناجح، مما أدي إلى ارتفاع الكلفة الاقتصادية على الأفراد والمجتمع.

الكلمات المفتاحية : القولون العصبي، اجتماع روما الثالث، الام البطن او المضايقة، الامراض الوظيفية للجهاز الهضمي.

*1 سالم محمد العوامي : salemawami@yahoo.com ، كلية الطب البشري، جامعة عمر المختار البيضاء – ليبيا.

Al-Mukhtar Journal of Sciences 33 (2): 104-111, 2018

The Effect of Storage Time and Different Anticoagulants on Fasting Blood Glucose Concentration

Khaled S. Al Salhen, Eman K. Saad and Aml J. Aznine

Department of Chemistry, Faculty of Science, Omar Al-Mukhtar University, El-Bayda, Libya

Received: 28 September / Accepted: 11 December 2017
Doi: https://doi.org/10.54172/mjsc.v33i2.173

Abstract: The comparative stabilizing effects of storage time and the anticoagulants; fluoride oxalate, EDTA, sodium citrate, and serum on ice slurry; on fasting blood glucose level were determined using the spectrophotometry method. Fasting blood samples were taken from 75 non-diabetic male people, and the blood glucose levels determined at 30 min intervals for a maximum time of 3 hours. Our results showed that the rate at which plasma glucose changes with time varies with specific anticoagulants. From the results, it was observed that the rate at which the blood glucose decreases with time vary with specific anticoagulants. It was noticed that random blood glucose in sodium citrate, EDTA, fluoride oxalate and serum on ice slurry decreased at a mean value of 28.4mg/dl, 58mg/dl, 15.4mg/dl and 60.2mg/dl dl after 3 hours respectively. With respect to the concentration of glucose before storage, this suggests that storage of blood using fluoride oxalate as an anticoagulant tends to better preserve the glucose level over a long period of time. Transport on ice slurry and rapid separation of serum within 30 min can inhibit glycolysis without the addition any anticoagulants (% reduction 1.3). Thus, from our findings, it is obvious that irrespective of the specimen type, time of collection or type of anticoagulant, the concentration of blood glucose remained unstable during storage. It is therefore suggested that analysis of blood glucose should be carried out immediately after collection of specimen or within the shortest possible time after storage in an anticoagulant to obtain a reliable result.

Keywords: anticoagulants, fasting blood glucose, glycolysis, sample collection.

INTRODUCTION

Most energy for cellular activities is derived from glucose, and more than 70% of the energy used by the human body is provided by the glucose oxidation process, which is important in maintaining the body's normal physiological functions (Zhu et al., 2017)). The blood sugar concentration or blood glucose level is the amount of glucose present in the blood of a human. Normally in mammals, the body maintains the blood glucose level at a reference range between 70 and 100 mg/dl before-meal (Baker et al., 1969). Glucose can be measured in whole blood, serum or plasma (Richard, 2001). Collection of blood specimen for measurement of blood glucose level should be done on the day and time requested. This is because collection times are related to food intake (Ochei and Kolhatkar, 2000). There are two different methods of determining glucose level: The chemical method and the enzymatic method. The chemical method exploits the non-specific reducing property of glucose in reactions with an indicator substance, which concomitantly changes color on its reduction (Louie et al., 2002). The enzymatic method has reached an

***Corresponding Author:** Khaled S. Al Salhen, email: khaled.alsalhen@omu.edu.ly Faculty of Science, Omar Al-Mukhtar University, El-Beida, Libya

advanced stage where the enzymes could be immobilized in electronic machines or devices for easier and faster analysis (Chernow et al., 1996). Glucose estimation using plasma or whole blood requires the use of an anticoagulant, which are compounds that help prevent the clotting of blood. When blood is shed or collected, the cell does not die immediately. They continue to metabolize and use up glucose as a source of energy via the glycolytic process. Glucose thus disappears from whole blood on standing over a period. Glycolysis can be prevented with an enzyme inhibitor (Lawrence et al., 2008).

The commonest inhibitor for this purpose is sodium fluoride, which usually used in conjunction with an anticoagulant potassium oxalate. Fluoride actually inhibits the enzyme enolase that found in the metabolic pathway of glucose and has a little effect on glucose oxidase and peroxidase enzymes. It also inhibits bacterial growth (Lawrence et al., 2008). Another widely used anticoagulant is Ethylene Diamine Tetra acetate (EDTA). When EDTA added to a blood sample, it chelates the calcium needed for blood clotting and thereby preventing the formation of fibrin. It forms an insoluble calcium salt by chelation. It was the purpose of this study to measure serum and plasma glucose concentration using different anticoagulants at different storage periods of time. Comparison of these anticoagulants helps to choose the suitable anticoagulant and to detect the effect of each anticoagulant at a specific storage time on serum and plasma glucose level. To our knowledge, no studies have been conducted on the investigation of the stability of blood glucose at different laboratory storage times and in different anticoagulant tubes in Libya.

MATERIALS AND METHODS

The study was carried out in University Clinic and biochemistry laboratory at Chemistry Department, Faculty of science, Omar Al-Mukhtar.

Study population and sample size: The study was covering 75 apparently healthy male individuals, randomly selected, and lived in El-Beida City from different age groups (49.7±11.45).

Exclusion criteria: Hemolytic samples and diabetic samples must be rejected.

Ethical consideration: All participants on this study were informed about the nature of study; blood samples were collected after their agreement.

Sample collection and processing: Vein side was cleaned with 70% alcohol; tourniquet was tied in space before collection. The needle was inserted and 6 ml of blood sample was collected. 2.0 ml from sample were applied to; fluoride oxalate anticoagulant container (Orange color), EDTA anticoagulant container (Green color), and sodium citrate (Pink Color) anticoagulant container and then were immediately separated at 3.000 rpm for 5 minutes at 20°C by using the centrifuge instrument. The serum was then separated from the blood cells in a plane container (Red color) and kept on an ice slurry. Blood samples were examined initially (at zero time), after 30, 60, 90, 120, and 180 minutes from sample collection.

Fasting blood glucose determination: The concentrations of fasting blood glucose in the plasma and serum were determined spectrophotometrically immediately after collection using Glucose-Oxidase test kit (Vitro Scient, Germany) described by (Werner et al., 1970). The procedure was repeated every 30 min interval for 3 h. Results were expressed as mg/dl.

Reference range of random blood glucose: Serum or plasma = 70-120 mg/dl (Trinder, 1969).

ISSN: online 2617-2186 print 2617-2178

Statistical analysis: The means and standard deviations (SD) were calculated and paired t-test was used to calculate P values.

RESULTS AND DISCUSSION

The assay of fasting blood glucose in samples stored in anticoagulants is a regular practice in this part of the world. When blood samples are collected, they are stored in their native state by preserving them in different anticoagulants. Though their native state is preserved, the blood glucose when assayed in different anticoagulants, at different times, varies. In this study, an attempt was made to compare the changes in blood glucose level over three hours at intervals of thirty minutes. From the results (Table 1), it was observed that the rate at which the blood glucose decreases with time vary with the specific anticoagulant.

Table (1). Effects of some anticoagulants on random blood glucose of some apparently healthy individuals.

Anticoagulant	Blood glucose concentration (mg/dl) during time (minutes)					
	Zero time	30	60	90	120	180
Sodium citrate	100.3±15.1	98.5±17. 7	92.6±21.7	87.1±23.1	78.9±25.2	71.9±29.0
EDTA	102.1±19.8	88.0±23.9	75.2±22.6	61.6±27.8	55.2±30.6	44.1±25.1
Fluoride oxalate	101.7± 23.6	100.9±12.6	100.1±16.1	99.4±12.9	98.1± 25.8	86.3± 29.6
Serum on ice slurry	100.9±22.4	99.5±22.8	79.9±27.2	65.6±29.1	50.1±33.2	40.7±35.4

Data are expressed as mean ± SD; n = 75; Ethylene Diamine Tetra Acetate (EDTA)

It was noticed that random blood glucose in sodium citrate, EDTA, fluoride oxalate and serum on ice slurry decreased at mean values of 28.4 mg/dl, 58mg/dl, 15.4 mg/dl and 60.2 mg/dl after 3 hours respectively. With respect to the concentration of glucose before storage, this suggests that storage of blood using fluoride oxalate as an anticoagulant tends to better preserve the glucose level over a long period of time. This may be due to the ability of fluoride ion to inhibit the activity of enolase, an enzyme in the glycolytic pathway, thereby slowing down the breakdown of glucose (Gupta & Kaur, 2014).

It can also be observed that irrespective of the anticoagulant used, the random blood glucose significantly ($P<0.05$) decreased steadily as compared to the value before storage. This actually shows that anticoagulants can not stop, in totality, the breakdown of glucose (glycolysis). Thus, over a long period of time, the concentration of glucose may reduce to zero level (Tables 2, 3, 4 and 5).

Table (2). Comparison between plasma glucose concentrations at zero with 30, 60, 90,120 and 180 minutes in sodium citrate anticoagulant.

Glucose concentration mg/dl ± SD at different times		
A	B	Mean differences(A-B)
Zero(100.3±15.1)	30 (98.5±17. 7)	1.8
Zero(100.3±15.1)	60 (92.6±21.7)	7.7
Zero(100.3±15.1)	90 (87.1±23.1)	13.2*
Zero(100.3±15.1)	120(78.9±25.2)	21.4*
Zero(100.3±15.1)	180(71.9±29.0)	28.4*

Data are expressed as mean ± SD; n = 75; *represents significant at *P<0.05* between different times.

Table (3). Comparison between plasma glucose concentrations at zero with 30, 60, 90,120 and 180 minutes in EDTA anticoagulant.

Glucose concentration mg/dl ± SD at different times		
A	B	Mean differences (A-B)
Zero(102.1±19.8)	30 (88.0±23.9)	14.2*
Zero(102.1±19.8)	60 (75.2±22.6)	26.9*
Zero(102.1±19.8)	90 (61.6±27.8)	40.5*
Zero(102.1±19.8)	120(55.2±30.6)	46.9*
Zero(102.1±19.8)	180(44.1±25.1)	58*

Data are expressed as mean ± SD; n = 75; *represents significant at *P<0.05* between different times.

ISSN: online 2617-2186 print 2617-2178

Table (4). Comparison between plasma glucose concentrations at zero with 30, 60, 90,120 and 180 minutes in fluoride oxalate anticoagulant.

Glucose concentration mg/dl ± SD at different times		
A	B	Mean differences (A-B)
Zero(101.7±23.6)	30(100.9±12.6)	0.8
Zero(101.7±23.6)	60(100.1±16.1)	1.6
Zero(101.7±23.6)	90(99.4± 12.9)	2.3
Zero(101.7±23.6)	120(98.1±25.8)	3.6
Zero(101.7±23.6)	180(86.3±29.6)	15.4*

Data are expressed as mean ± SD; n = 75; *represents significant at *P<0.05* between different times.

It was noticed that fasting blood glucose in sodium citrate, EDTA, fluoride oxalate and serum on ice slurry decreased at mean percentage values of 28.4%, 56.8%, 13.7% and 59.6% after 3 h (Table 6).

Table (5). Comparison between serum glucose concentrations on ice slurry at zero with 30, 60, 90,120 and 180 minutes.

Glucose concentration mg/dl ± SD at different times		
A	B	Mean differences (A-B)
Zero(100.9±22.4)	30 (99.5±22.8)	1.4
Zero(100.9±22.4)	60 (79.9±27.2)	21*
Zero(100.9±22.4)	90 (65.6±29.1)	35.3*
Zero(100.9±22.4)	120(50.1±33.2)	50.8*
Zero(100.9±22.4)	180(40.7±35.4)	60.2*

Data are expressed as mean ± SD; n = 75; *represents significant at *P<0.05* between different times.

Table (6). Comparison between reduction percentage of serum and plasma glucose levels in different anticoagulants.

Anticoagulant	Mean of % reduction from zero to 30 min	Mean of % reduction from zero to 60 min	Mean of % reduction from zero to 90 min	Mean of % reduction from zero to 120 min	Mean of % reduction from zero to 180 min
Sodium citrate	1.7	7.6	13.1	21.1	28.1
EDTA	13.8	26.3	39.6	45.9	56.8
Fluoride oxalate	0.78	1.57	2.2	3.5	13.7
Serum on ice slurry	1.3	20.8	34.9	50.3	59.6

These also support the possibility for fluoride oxalate to be a better anticoagulant for long-term storage of blood samples for glucose determinations, since the glucose concentration in the blood samples stored in it tends to be comparatively more stable (% reduction 13.7 after 3 h).(Chan et al., 1989)) reported that antiglycolytic action of fluoride is delayed for up to 4 h and has little or no effect on the rate of glycolysis during the first 1-2 h after blood is collected. Glucose levels can fall as much as 10 mg/dl during this period. Transport on ice slurry and rapid separation of serum within 30 min can inhibit glycolysis without the addition of any anticoagulants (Table 6; % reduction 1.3), and in fact works better as shown by few studies (Gambino, 2013; Waring et al., 2007).

The recommendations of American Diabetes Association (ADA) published in 2002 and WHO guidelines of 2006 clearly indicated that venous plasma is the preferred sample for glucose estimation (Organization, 2006; Sacks et al., 2002). However in most laboratory panels, serum is the most suitable sample for all other chemistries performed, and so ‘‘panel’’ glucose is usually serum glucose. The requirement that serum samples must be allowed to clot before serum glucose is tested and significantly increases turnaround time for glucose results compared with plasma results(Schrot et al., 2007). There is also a suggestion that clotting consumes glucose (Gambino et al., 2009). Therefore, serum glucose concentrations will always be lower than plasma glucose if glycolysis in a plasma sample is inhibited immediately. The amount of the differences will vary with the glycolysis rate in the individual specimen and the time elapsed between collection and centrifugation(Gambino, 2013). The comparison of paired blood samples for serum and heparinized plasma collected and

ISSN: online 2617-2186 print 2617-2178

stored at same ambient temperature and centrifuged at same time produced higher glucose values in plasma (Gambino et al., 2009). On the other hand, studies comparing the results of serum gel separator tubes with those of fluoride tubes have reported higher glucose values in serum samples (Turchiano et al., 2013). This is because when serum is collected in tubes with a clot activator, serum gel separator, and promptly centrifuged, glycolysis is stopped quickly due to the separation of serum from the cellular components (Turchiano et al., 2013). The use of serum for glucose estimation is not uncommon in the world. This means that the practice of using serum sample for glucose estimation could be leading to many wrong reports and responsible for false variation in results of an individual obtained from different laboratories as well as misclassification of at risk patients (Gupta & Kaur, 2014). But WHO and ADA have emphasized on the need of putting the plasma glucose samples immediately on ice slurry and centrifugation within 30 min (Sacks et al., 2011).

This may be difficult but not impossible to achieve as a short-term measure for accurate reporting of glucose levels. Mechanisms can be developed to either centrifuge the glucose samples immediately in the sample collection area or transport to central lab on ice packs or they can be directly sent to laboratory on priority as is immersed in ice slurry, where immediate processing could take place (Gupta & Kaur, 2014).

As observed in Tables 2, 3 and 4, they also showed significantly decrease ($P<0.05$) in fasting blood glucose levels in the blood samples stored in all the anticoagulants under study. Thus, from our findings, it is obvious that irrespective of the specimen type, time of collection or type of anticoagulant, the concentration of blood glucose remained unstable during storage. It is therefore suggested that analysis of blood glucose concentrations should be carried out immediately after collection of the specimen or within the shortest possible time after storage in an anticoagulant, so as to obtain a reliable result.

CONCLUSION

From our findings, it is obvious that irrespective of the anticoagulant used, time of collection of specimen or type of specimen, the concentration of glucose is never stable. Thus, to get reliable results, glucose determination should be carried out immediately after collection of samples or within the shortest possible time. Fluoride oxalate is more stable anticoagulant than the other anticoagulants. Reduction percentage was more pronounced between 0-3 hours. For this reason, it is better to measure the concentration of fasting glucose within the shortest time after sample collection.

REFERENCES

Baker, L., Root, A. W., Haque, N., & Kaye, R. (1969). Metabolic homeostasis in juvenile diabetes mellitus. I. Role of growth hormone. *Metabolism, 18*(2), 110-114 .

Chan, A., Swaminathan, R., & Cockram, C. (1989). Effectiveness of sodium fluoride as a preservative of glucose in blood. *Clinical Chemistry, 35*(2), 315-317 .

Chernow, B., Jackson, E., Miller, J., & Wiese, J. (1996). Blood conservation in acute care and critical care. *AACN Advanced Critical Care, 7*(2), 191-197 .

Gambino, R. (2013). Sodium fluoride: an ineffective inhibitor of glycolysis. *Annals of clinical biochemistry, 50*(1), 3-5 .

Gambino, R., Piscitelli, J., Ackattupathil, T. A., Theriault, J. L., Andrin, R. D., Sanfilippo, M. L., & Etienne, M. (2009). Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. *Clinical Chemistry, 55*(5), 1019-1021 .

ISSN: online 2617-2186 print 2617-2178

Gupta, S., & Kaur, H. (2014). Inhibition of glycolysis for glucose estimation in plasma: recent guidelines and their implications. *Indian Journal of Clinical Biochemistry, 29*(2), 262-264 .

Lawrence, J. M., Contreras, R., Chen, W., & Sacks, D. A. (2008). Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005. *Diabetes care, 31*(5), 899-904 .

Louie, L., Goodfellow, J., Mathieu, P., Glatt, A., Louie, M., & Simor, A. (2002). Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. *Journal of clinical microbiology, 40*(8), 2786-2790 .

Organization, W. H. (2006). What diagnostic tests should be used to define glycemic status. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Geneva: WHO*, 29-30 .

Sacks, D. B., Arnold, M., Bakris, G .L., Bruns, D. E., Horvath, A. R., Kirkman, M. S., Lernmark, A., Metzger, B. E., & Nathan, D. M. (2011). Position statement executive summary: guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Diabetes care, 34*(6), 1419-1423 .

Sacks, D. B., Bruns, D. E., Goldstein, D. E., Maclaren, N. K., McDonald, J. M., & Parrott, M. (2002). Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clinical Chemistry, 48*(3), 436-472 .

Schrot, R. J., Patel, K. T., & Foulis, P. (2007). Evaluation of inaccuracies in the measurement of glycemia in the laboratory, by glucose meters, and through measurement of hemoglobin A 1c. *Clinical Diabetes, 25*(2), 43-49 .

Turchiano, M., Nguyen, C., Fierman, A., Lifshitz, M., & Convit, A. (2013). Impact of blood sample collection and processing methods on glucose levels in community outreach studies. *Journal of environmental and public health, 2013* .

Waring, W., Evans, L., & Kirkpatrick, C. (2007). Glycolysis inhibitors negatively bias blood glucose measurements: potential impact on the reported prevalence of diabetes mellitus. *Journal of clinical pathology, 60*(7), 820-923 .

Werner, W., Rey, H., & Wielinger, H. (1970). Properties of a new chromogen for determination of glucose in blood according to god/pod-method. *Zeitschrift fur Analytische Chemie Fresenius, 252*(2-3), 224 .+-

Zhu, X.-L., Wang, S.-H., Cui, Y.-X., Yao, R.-F., Chen, J., & Jin, H.-Z. (2017). Effect of specimen storage time on neonatal blood glucose level. *Biomedical Research, 28*(14 .(

Baker, L., Root, A. W., Haque, N., & Kaye, R. (1969). Metabolic homeostasis in juvenile diabetes mellitus. I. Role of growth hormone. *Metabolism, 18*(2), 110-114 .

Chernow, B., Jackson, E., Miller, J., & Wiese, J. (1996). Blood conservation

ISSN: online 2617-2186 print 2617-2178

in acute care and critical care. *AACN Advanced Critical Care, 7*(2), 191-197 .

Gupta, S., & Kaur, H. (2014). Inhibition of glycolysis for glucose estimation in plasma: recent guidelines and their implications. *Indian Journal of Clinical Biochemistry, 29*(2), 262-264 .

Lawrence, J. M., Contreras, R., Chen, W., & Sacks, D. A. (2008). Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005. *Diabetes care, 31*(5), 899-904 .

Louie, L., Goodfellow, J., Mathieu, P., Glatt, A., Louie, M., & Simor, A. (2002). Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. *Journal of clinical microbiology, 40*(8), 2786-2790 .

Werner, W., Rey, H., & Wielinger, H. (1970). Properties of a new chromogen for determination of glucose in blood according to god/pod-method. *Zeitschrift fur Analytische Chemie Fresenius, 252*(2-3), 224 .+-

Zhu, X.-L., Wang, S.-H., Cui, Y.-X., Yao, R.-F., Chen, J., & Jin, H.-Z. (2017). Effect of specimen storage time on neonatal blood glucose level. *Biomedical Research, 28*(14 .(

Baker, L., Root, A. W., Haque, N., & Kaye, R. (1969). Metabolic homeostasis in juvenile diabetes mellitus. I. Role of growth hormone. *Metabolism, 18*(2), 110-114 .

Chernow, B., Jackson, E., Miller, J., & Wiese, J. (1996). Blood conservation in acute care and critical care. *AACN Advanced Critical Care, 7*(2), 191-197 .

Lawrence, J. M., Contreras, R., Chen, W., & Sacks, D. A. (2008). Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005. *Diabetes care, 31*(5), 899-904 .

Louie, L., Goodfellow, J., Mathieu, P., Glatt, A., Louie, M., & Simor, A. (2002). Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. *Journal of clinical microbiology, 40*(8), 2786-2790 .

Werner, W., Rey, H., & Wielinger, H. (1970). Properties of a new chromogen for determination of glucose in blood according to god/pod-method. *Zeitschrift fur Analytische Chemie Fresenius, 252* .+-224 ،(3-2)

Zhu, X.-L., Wang, S.-H., Cui, Y.-X., Yao, R.-F., Chen, J., & Jin, H.-Z. (2017). Effect of specimen storage time on neonatal blood glucose level. *Biomedical Research, 28*(14).

ISSN: online 2617-2186 print 2617-2178

تأثير وقت التخزين ومضادات التخثر المختلفة على مستوى السكر في الدم

خالد الصالحين*، إيمان سعد وأمل ازنين

قسم الكيمياء، كلية العلوم، جامعة عمر المختار، البيضاء –ليبيا

تاريخ الاستلام: 28 سبتمبر 2017 / تاريخ القبول: 11 ديسمبر 2017
Doi:https://doi.org/10.54172/mjsc.v33i2.173

المستخلص : دراسة تأثير زمن التخزين على مستوى الجلوكوز في عينة دم مخزنة في مضادات تجلط مختلفة من أوكسالات الفلوريد، إديتا (EDTA)، سيترات الصوديوم وعينة سيرم في ثلج مجروش وذلك باستخدام طريقة الطيف الضوئي. أخذت عينات الدم من 75 ذكراً غير مصابين بمرض السكري، وحددت مستويات السكر في الدم على فترات 30 دقيقة لمدة أقصاها ساعتان، وأظهرت النتائج أن المعدل الذي يتغير فيه جلوكوز البلازما مع مرور الوقت يختلف مع اختلاف مضادات التخثر. لوحظ أن المعدل الذي ينخفض فيه الجلوكوز في الدم مع مرور الوقت يختلف مع اختلاف مضادات التخثر، كما أن الجلوكوز في الدم العشوائي في سيترات الصوديوم وإديتا (EDTA) وأوكسالات الفلوريد والمصل في الثلج المجروش قد انخفض بمعدل متوسط قدره 28.4مليجرام/ دل، 15.4 مليجرام/ دل و60.2 مليجرام/ دل بعد مرور 3 ساعات على التوالي. فيما يتعلق بتركيز الجلوكوز قبل التخزين، يشير هذا إلى أن تخزين الدم باستخدام أوكسالات الفلوريد كمضاد للتخثر، يميل إلى الحفاظ على مستوى الجلوكوز بشكل أفضل على مدى فترة طويلة من الزمن. التخزين على الثلج المجروش والفصل السريع من المصل في غضون 30 دقيقة يمكن أن تمنع التحلل دون إضافة أي مضادات للتخثر (٪ تخفيض 1.3). يمكننا أن نستنتج بغض النظر عن نوع العينة، ووقت جمع أو نوع مضادات التخثر، فإن تركيز الجلوكوز في الدم سيبقى غير مستقر أثناء فترة التخزين الطويلة، ولذلك يقترح إجراء تحليل لجلوكوز الدم مباشرة بعد جمع العينات أو في أقصر وقت ممكن بعد التخزين في مضاد للتخثر، وذلك للحصول على نتيجة يمكن الاعتماد عليها.

الكلمات المفتاحية : مضادات التخثر، مستوى الجلوكوز في الدم، تحلل الجلوكوز، جمع العينات.

* خالد الصالحين khaled.alsalhen@omu.edu.ly قسم الكيمياء، كلية العلوم، جامعة عمر المختار

Al-Mukhtar Journal of Sciences 33 (2): 112-116, 2018

Al-Jala Hospital Experience on Total Knee Arthroplasty

Salah Bayio[1] and Ramzi Ahmed Hussein*[2]

[1]*Department of Orthopedics and Traumatology, faculty of Medicine, Benghazi University - Libya*
[2]*Department of orthopedic, Faculty of Medicine, Omar Al-Mukhtar University, El-Bayda, Libya*

Received: 5 October 2017 / Accepted: 18 February 2018
Doi: https://doi.org/10.54172/mjsc.v33i2.174

Abstract: The aim of this Prospective study was to analyze the results and our experience in the knee Arthroplasty for the management of primary osteoarthritis at aljala teaching hospital in Benghazi. From January 2007 to December 2008, our orthopedic department; male orthopedic A (MOA) and female orthopedic word (FOW) operated 60 knees of 55 patients. 43 of them were females (78%) and 12(22%) were males, and the mean age was 56 years. We recorded patient's details; age, sex, Right or Left knee days of staying in hospitalization, Classification of OA [primary or secondary], time of operation, and associated diseases [hypertension, Diabetes mellitus, Ischemic heart disease]. A physical examination to assess knee motion, stability, strength, and overall leg alignment and X-rays (radiographs) to determine the extent of damage and deformity of the knee. Post-operative complications and Revision. At final evaluation after a minimum period of 3 years; 5(9%) patients had bilateral total knee replacement, 32 right knee patients (58%), and 23left knee patients (42%). Loosening tibial component was found in two patients (3.6%), and one patient (1.8%) was complicated by postoperative infection. Patients with rheumatoid arthritis showed more improvement than those with osteoarthritis. The conclusions with regards to the differential treatment of women are almost two-thirds as likely to undergo a TKA as men. Elective TKA is an effective treatment option for end-stage osteoarthritis of the knee.

Keywords: Arthroplasty**,** Osteoarthritis**,** Total knee arthroplasty.

INTRODUCTION

Total knee replacement (TKR), also referred to as total knee arthroplasty (TKA), is a surgical procedure where worn, diseased, or damaged surfaces of a knee joint are removed and replaced with artificial surfaces. Materials used for resurfacing of the joint are not only strong and durable but also optimal for joint function as they produce as little friction as possible. The "artificial joint or prosthesis" generally has two components, one made of metal which is usually cobalt-chrome or titanium. The other component is a plastic material called polyethylene [figure 1]. Modern knee arthroplasty began in the early 1970s with the development of the total condylar knee prosthesis. Survivorship studies with this prosthesis are the standard with which modern knee replacement is compared. Long-term series by Ranawat *et al.* Font-Rodriguez *et al.* and Pavone et al. have documented the longevity of the original total condylar prosthesis to be 95% at 15 years and 91% at 21 and 23 years(Font-Rodriguez et al., 1997; Ranawat, 1986)

***Corresponding Author:** Ramzi Ahmed Hussein mahjob777@yahoo.com Faculty of medicine, Omar Al-Mukhtar University, El-Beida, Libya

Figure (1). Components of knee arthroplasty

Cementless fixation has had mixed results with respect to prosthesis survivorship. Some designs have equaled the success of cemented designs whereas others have had higher rates of failure because of tibial loosening, polyethylene wear, and osteolysis (Murray et al., 1994; Whaley et al., 2003). The general goal of total knee replacement is to provide painless and unlimited standing, sitting, walking, and other normal activities of daily living, but it is not designed for sports like tennis.

MATERIALS AND METHODS

Data were collected for all attendances over the years (2007-2008) in which the cases with primary osteoarthritis treated by TKR at Al-jalla teaching hospital (Orthopedic Department –Benghazi) main regional trauma centre. We recorded patient's details: Age, sex, side, days of staying in hospitalization, classification of O.A [primary or secondary], time of operation, associated diseases [Ahypertension–Diabetes mellitus– Ischemic heart disease], a physical examination to assess knee motion, stability, strength, and overall leg alignment and X-rays (radiographs) to determine the extent of damage and deformity of the knee, post operative complications, revision done or not.

Our treatment protocol included the routine investigation of hemoglobin levels, blood sugar, blood group, renal function, urine examination, electrocardiograph and chest radiography, and any medical problems were treated with the help of physician and anesthetist so the surgery could be carried out as soon as possible. We used the cementing techniques in all surgical procedures, and the healing process was determined both clinically and radio graphically [figure 2].

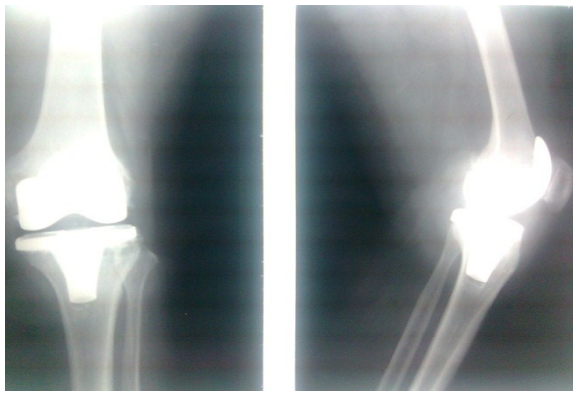


Figure (2). X-Ray of TKR

Statistics: From January 2007 to December 2008, our unit operated (60) knees of (55) patients with primary osteoarthritis admitted from the total number of admission (3509) to MOA-FOW. In 2007 (688) patients admitted to F.O.W and (1021) patients admitted to M.O.W. In 2008 (809) patients admitted to F.O.W and (991) patients admitted to M.O.W

Post-operative policy: Sciatic nerve block may provide earlier anesthesia effects than Local infiltration anesthesia when combined femoral nerve block. However, there were no differences in morphine use, active knee flexion, postoperative nausea, and vomiting between the groups. The Local infiltration anesthesia group spent less time under anesthesia, suggesting that Local infiltration anesthesia may offer a practical and potentially safer alternative to Sciatic nerve block (Li *et al,.* 2016). We started the isokinetic physiotherapy in the second week in the non-complicated cases by using the continuous passive motion machine (Mclnnes et al., 1992) and were discharge after two

ISSN: online 2617-2186 print 2617-2178

weeks from the admission exept for one patient who stayed for 6 weeks to control post operative infection (Schoifet & Morrey, 1990).

Follow-up: patients were normally reviewed in the first week, third week, and every month until healing occurred. The patients have a follow up protocol reaching up to 3 years (36 month). Compared to 45 months for WOMAC (Western Ontario and McMaster Universities). However, weighting for baseline knees and Knee Society score (KS) had a mean follow up of 90 months and 68 months for WOMAC, but Hospital for Special Surgery scale (HSS) was only 61 months. The longest mean follow up time was 90 months (KS scores weighted for baseline knees), way less than the 10 years that has been suggested in order to evaluate long term functional results (Crowder et al., 2005; Insall et al., 1989)

RESULTS

In average, the patients were approximately 56 years of age and very few of them were over the age of 75 [tab.1]. nearly all of the patients had primary osteoarthritis, and about 78% were females and 22% were males (figure 3).

Table (1). Characteristic of the 55 patients.

Male to female ratio	12 male -43 female
Age of the patient (Average)	56 years
Body weight (Average)	80 kg
Bilateral TKR	5 patients
Right knee Replacement	32 patients
Left knee Replacement	23 patients
Patients with rheumatoid arthritis	3 patients
Range of motion post O.T	90°
Loosening tibial component	2 patients
Infection Post Operative	One patient

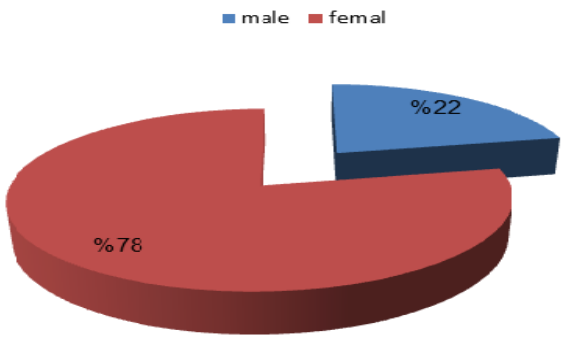


Figure (3). male and female ratio

Four patients presented with HTN and D.M, three patients with IHD, renal stone one patient, and 3 patients with kwon case of rheumatoid arthritis and one of them was 52 years female also presented with HTN and D.M operated and followed up during the period of study with no complication. The average time to healing of the 60 total knee replacement was 4 months (minimum 3 months, maximum 7 months). The overall knee range of motion averaged 90°. Body weight average 80 kg, Bilateral total knee replacement was applied for 5 patients (9%), right knee 32 (58 %), and left knee 23patients (42 %) (figure 4).

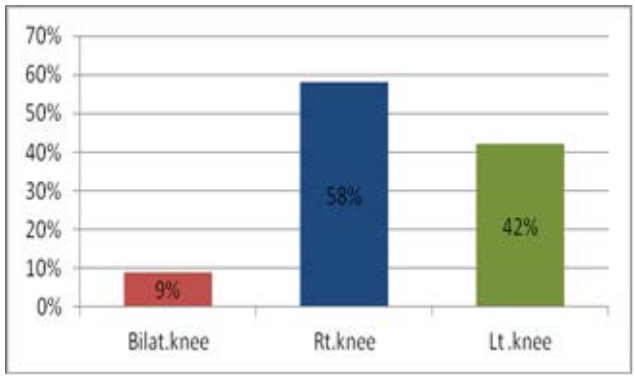


Figure (4). patients knee ratio for arthroplasty

Loosening tibial component was found in two patients, one female was 58 years (D.M and HTN) and the other female was 60 years (HTN and IHD). One female patient complicated by post operative infection was 65 years (CRP was 311 .. 78) and was planning for revision.

ISSN: online 2617-2186 print 2617-2178

DISCUSSION

Patients with rheumatoid arthritis showed more improvement than those with osteoarthritis, but this may be related to their poorer functional scores at the time of treatment and hence the potential for more improvement. Elective total knee Arthroplasty is an effective treatment option for end-stage osteoarthritis of the knee. The strongest evidence exists over a follow up period of up to two years, but the studies that extend to 5 and even 10 years of follow up show positive results as well (Crowder et al., 2005).

The revision rate through two or more years is 1.8 percent of knees and 1.6 percent of patients. Postoperative complications occurred in 1.8 percent of patients and 1.6 percent of knees. There were only 11 patients with cardiovascular or renal complications . The conclusions with regard to the differential treatment of women are almost two-thirds as likely to undergo a TKA as men. These conclusions are tempered by the limitations of the designs of many studies included in the analysis. Although osteoarthritis does not seem to be a predictor of outcomes, the results seem to be somewhat better for rheumatoid arthritis(Alicea, 2001; Bellamy et al., 1988).

REFERENCES

Alicea, J. (2001). Scoring systems and their validation for the arthritic knee. *Surgery of the knee, 2*, 1507-1515 .

Bellamy, N., Buchanan, W. W., Goldsmith, C. H., Campbell, J., & Stitt, L. W. (1988). Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *The Journal of rheumatology, 15*(12), 1833-1840 .

Crowder, A. R., Duffy, G. P., & Trousdale, R. T. (2005). Long-term results of total knee arthroplasty in young patients with rheumatoid arthritis. *The Journal of arthroplasty, 20*, 12-16 .

Font-Rodriguez, D. E., Scuderi, G. R., & Insall, J. N. (1997). Survivorship of cemented total knee arthroplasty. *Clinical orthopaedics and related research, 345*, 79-86 .

Insall, J. N., Dorr, L. D., Scott, R. D., & Scott, W. N. (1989). Rationale of the Knee Society clinical rating system. *Clin Orthop relat res, 248*(248), 13-14 .

Mclnnes, J., Larson, M. G., Daltroy, L. H., Brown ‹T., Fossel, A. H., Eaton, H. M., Shulman-Kirwan, B., Steindorf, S., Poss, R., & Liang, M. H. (1992). A controlled evaluation of continuous passive motion in patients undergoing total knee arthroplasty. *Jama, 268*(11), 1423-1428 .

Murray, P. B., Rand, J .A., & Hanssen, A. D. (1994). Cemented long-stem revision total knee arthroplasty. *Clinical orthopaedics and related research, 309*, 116-123 .

Ranawat, C. S. (1986). The patellofemoral joint in total condylar knee arthroplasty: pros and cons based on five-to ten-year follow-up observations. *Clinical orthopaedics and related research, 205*, 93-99 .

Schoifet, S., & Morrey, B. (1990). Treatment of infection after total knee arthroplasty by débridement with retention of the components. *JBJS, 72*(9), 1383-1390 .

Whaley, A. L., Trousdale, R. T., Rand, J. A., & Hanssen, A. D. (2003). Cemented long-stem revision total knee arthroplasty. *The Journal of arthroplasty, 18*(5), 592-599 .

ISSN: online 2617-2186 print 2617-2178

خبرة مستشفى الجلاء في المفصل الصناعي للركبة

صلاح بعيو[1] ورمزي احمد الحاسي[2]

[1] قسم جراحة العظام والكسور، كلية الطب البشري، جامعة بنغازي – ليبيا

[2] قسم جراحة العظام، كلية الطب البشري ، جامعة عمر المختار ، البيضاء – ليبيا

تاريخ الاستلام: 5 اكتوبر 2017/ تاريخ القبول :18 فبراير 2018

Doi:https://doi.org/10.54172/mjsc.v33i2.174

المستخلص: المفصل الصناعي للركبة عبارة عن عملية جراحية يتم فيها استبدال الركبة التالفة أو المصابة بمرض جفاف المفاصل (الفصال العظمي) بمفصل صناعي لا يتميز بالصلابة والثبات وحسب بل يؤدي وظيفة الركبة الطبيعية. والهدف من البحث دراسة تحليلية لعدد من المرضى المصابين بمرض الفصال العظمي للركبة خلال الفترة الممتدة من شهر يناير 2007 م إلى نهاية شهر ديسمبر 2008 م، حيث قمنا بدراسة تحليلية للركب المستبدلة للحالات المرضية التي دخلت مستشفى الجلاء للحوادث بنغازي خلال تلك الفترة. الدراسة شملت عدد 55 مصاباً متوسط أعمارهم 56 سنة وبعض الحالات كانت مصابة بأمراض مزمنة مثل مرض السكري والضغط وكذلك بعض الحالات كانت مصابة بمرض التهاب المفاصل الروماتويدي، وبعد إجراء التحاليل والأشعة اللازمة تم استبدال 60 مفصلاً صناعياً لعدد 55 مريضاً، من الرجال 12 مريضاً والنساء 43 مريضة ومن بين هذه الحالات 5 مرضى قمنا باستبدال الركبتين على مرحلتين، وخلال فترة المتابعة ولمدة ثلاث سنوات كانت النتائج النهائية للدراسة حدوث التهاب المفصل الصناعي لمريض واحد فقط، أما ارتخاء أو تفكك المفصل الصناعي فكان لمريضين. وكانت النتائج مرضية لمرضى الروماتويد وللنساء أكثر من الرجال، المفصل الصناعي للركب يعتبر من العلاجات الناجحة للمرضى المصابين بمرض الفصال الركبي.

الكلمات المفتاحية: المفصل الصناعي للركبة – الفصال الركبي – المفصل الصناعي.

*رمزي احمد الحاسي mahjob777@yahoo.com كلية الطب ، جامعة عمر المختار ، البيضاء

Al-Mukhtar Journal of Sciences 33 (2): 117-123, 2018

Foraging Behaviour of Honey Bees *Apis mellifera* Linn. Visiting The Flowers of Some Wild Plants in Eljabal Alakhder-Libya

Ali A. Bataw* **and Nesrin K. Shareef**

Department of Zoology, Faculty of Science – Omar Al-Mukhtar University, Al-Bayda, Libya

Received: 16 December 2017 / Accepted: 11 April 2018
Doi: https://doi.org/10.54172/mjsc.v33i2.175

Abstract: This study was conducted to identify the foraging behaviour of honey bees *Apis mellifera* in the search for food during their visit to the wild flower plants *Sinapis alba*, *Pelargonium radula*, *Malva parviflora* and *Stachy stournefortii* in Eljabal Alakhder region. The results showed differences in the handling time periods with a significant difference between plant flower species. It showed a longer resting period compared with the handling time for flowers of the *Pelargonium radula*, which recorded the lowest time, also the travelling time of honey bee among the flowers of the plant species showed a significant difference. *Stachy stournefortii* recorded a longer travelling time with an average of 4.3 seconds, and *Pelargonium radula* with 3.5 seconds. *Apis mellifera* showed a different activity among the different flowers in the collection of nectar or pollen during different daytime hours.

Keywords: Foraging behaviour, Libya, *Apis mellifera*, wild flowers, Eljebal Alakhder

INTRODUCTION

Foraging activity is measured by using different parameters including, the foraging commencement or/and cessation time (Joshi & Joshi, 2010; Mattu et al., 2012; Tan et al., 2012). Other parameters related to foraging activity and the visiting of plants include the number of foragers per flower and time spent per flower (Sushil et al., 2013); nectar and pollen collection method from the blooms (MacKenzie, 1994); the proportion of pollen or nectar foragers relative to total foragers; foraging type; the load of pollen and pollen type; concentration of crop nectar sucrose (Riddell Pearce et al., 2013). The resting time of the bee on the flower is the time spent from the moment it descends to the moment when it left the flower, and the travelling time is known at the time it takes for bees to travel from a flower to another of the same plant (Steel et al., 1980). Herrera (1989) noticed that there was a relationship between the length of the bee's mouth and the length of its resting time on the flower. Long-tongued species such as *Anthophora quadrifasciata*, whose length of mouth parts was 11.1 mm long, had a resting time of 0.8 seconds, while the honey bees which has a length of 5.1 mm of her mouth parts, had a resting time of 3.6 seconds and that indicates that the longer the bees' mouths are, the shorter the length of time needed on the flower, and (Willmer et al., 1994) confirmed that the resting time of honeybees that visited the flower of Glen Clova is 11.53 seconds, Glen Prosen 10.49 seconds, and 21.42 seconds on the Glen Moy flowers. Also, the length of travelling time in flowers is different from one plant to another. For the flower of Glen Clova it was 3.27 seconds, 4.35 seconds on Glen Moy, and 4 seconds on the flower of the plant Glen Prosen. Bataw and Lamin (2001) also noticed that the resting time length of honeybees on *Rosmarinus officinalis* was 1.32 seconds, and

***Corresponding Author:** Ali A. Bataw, ali.bataw@omu.edu.ly Faculty of Science,Omer Al - Mukhtar University, Al-Bayda, Libya

the travelling time was 1.5 seconds. Gegear and Laverty (2004) assessed the flower constancy of *Apis mellifera* and *Bombu impatiens* during the visit to two types of flowers (yellow flowers- blue flowers), the study showed that the honey bee has a high stability on one type of the flowers and took a longer time in the movement between flowers, and the length of resting time did not differ significantly between the two types. (Fahn & Shimony, 2001) who worked on *Lysioglossum spp* and showed that honeybees spend a long resting period on *Ecballium spp*. while the *Ceratina* bees showed a short resting time on the same flowers. The difference in the resting and travelling time changes according to the type of plant and reward sponsored by the bees, and the length of time bees stand on the flowers when visiting depends on the type and quantity of nectar and pollen (Harder, 1986). YeboahGyan and Woodell pointed out in 1987 that honeybees collected pollen from the flowers of the plant of *Rubus fruticusus* in the early morning and this may be due to the low concentration of sugar in the nectar because of the high humidity and generally; collected pollen by bees increased during the day and extend to what between noon and afternoon. (Corbet & Delfosse, 1984) noticed that honeybees collect pollen of *Echium pgantagineum* only when the concentration of nectar is less than 35%. In a study conducted by Sazima and Feritas (2003) on the flower of *Viola spp,* they pointed out that the primary pollinator of the flowers of this plant are females belonging to bees *Andrena spp*, which are mainly looking at the pollen by shaking the flower, and they pointed out that the males of this species circled around the flower clusters to feed on nectar and represent secondary pollinators. (Giurfa & Núñez, 1992) concluded that honeybees used the smell of visited flowers to avoid the lack of content of the nectar. Nectar volume and concentration are the basis upon which nectar energetics are calculated, and the abundances of the dominant species of flower visitors within some ecosystems are linked to the amount of energy provided by the nectar (Roubik 1989). Daily changes in available nectar clearly affect the identity and abundance of flower feeders (Potts *et al*. 2001; 2004). Relatively, a little is known about the honey bee foraging behavior in Aljabal Alakhder. Our objective was to establish a baseline foraging behavior to aid in establishing long-term monitoring pollinator programs.

MATERIALS AND METHODS

The study site was an area near the buildings of Omar Elmukhtar University,(32°45’14 N21°42’42 E; altitude 6255 m) in Albaida, Libya. The experemint was carried out during the flowering season period of the wild plants*, Sinapis alba*, *Pelargonium radula*, *Malva parviflora*, and *Stachy stournefortii*. These species are among the common wild plant flowers in the area and their flowering season extends from February until the month of August. Their flowers provide an important source of food for honeybees, where many beekeepers keep their bee hives near these areas.

Rate of food search: The time spent on a single flower was calculated once a bee touches any part of the flower (*Handling time*), as well as the time it traveled from one flower to another during the flowering season (*Travelling time*). Readings were taken during the period from 8:00 to 13:00 using a stopwatch in the same way as previously described ((Pleasants, 1981).

Nectar and pollen collection times: The behavior of honeybee workers was monitored during their visits to plant flowers, the number of visiting workers, and the times when nectar was collected by extending their mouths inside the flower or collecting the pollen through the use of its front legs from 8:00 to 13:00.

Statistical analysis: Statistical analysis of all

ISSN: online 2617-2186 print 2617-2178

data were obtained by using Minitab (16), One-way ANOVA, mean and standard error (±SE) using the Tukeys' method.

RESULTS

Period of foraging: The results showed a clear variation in handling time with the visits of bees to the different plant flowers (One- way ANOVA) : df = 3, F = 7.72, P <0.005). The *Apis mellifera* has a longer handling time on the flowers of *Sinapis alba*, which was 8.9 seconds, compared to the flowers of *Pelargonium radula*, which had a handling time of 7.2 seconds, while on the flowers of *Malva parviflora* had a period of 8.4 seconds, and on the flowers of *Stachy stournefortii* recorded 8.6 seconds. The results also revealed that there was a significant difference in the travelling time between the flowers of different plant species (One-way ANOVA : df = 3, F = 22.80, P <0.005). The *Stachy stournefortii* flower recorded a longer travelling time of 4.3 seconds while the *Sinapis alba* flower recorded the lowest travelling time of 2.8 seconds. *Malva parviflora* flowers recorded an average of travelling time of 3.7 seconds and the flower of *Pelargonium radula* recorded a travelling time of 3.5 seconds.(Table 1).

Table (1). Mean (±SE) of the handling time of the *Apis mellifera* on the flower and the travelling time (in sec.) between different flower species.

Plant flowers	Handling time (sec.)	Travelling time (sec.)
Sinapis alba	8.9± 0.319 [c]	2.8± 0.086 [c]
Pelargonium radula	7.2± 0.369 [b]	3.5± 0.135 [b]
Malva parviflora	8.4± 0.201 [a]	3.7± 0.113 [b]
Stachy stournefortii	8.6± 0.307 [a]	4.3± 0.137 [a]

Similar letters mean that there is no significant difference within each column (P <0.005)

Foraging Behavior of bees in the collection of nectar and pollen :. By observing the foraging behaviour of *Apis mellifere* during their visits to the different flower species (Table 2), We noticed on the flowers of *Stachy stournefortii* that the workers collected nectar and pollen by entering their head through the opening of the corolla and extending their mouth to the bottom of the flower to suck the nectar, and the rest of the body was out and by using the help of legs to hold on the flower. The results showed that the highest percent number of workers (75.5%) collecting nectar was recorded at 10 am, and the lowest number of workers (36.9%) collecting nectar was at 13:00 and we recorded a higher percentage number of bees collecting pollen at 13:00 with 63.1%, and the lowest percentage number was (24.5%) at 10:00 The honey bee, *Apis mellifera*, on the flowers of *Sinapis alba*, was active in collecting pollen only from 8:00 am to 13:00 by 100%. It was noticed that it moves its front legs when it stands on the flower. Honey bees also showed the same behaviour during their visit to the flowering plant *Malva parviflora* by collecting pollen grains with 100% starting from 8:00 am to 13:00. The honey bees workers showed a different activity on the flowers of the *Pelargonium radula*, by collecting nectar only from 8:oo to 13:00 at 100%.

ISSN: online 2617-2186 print 2617-2178

Table (2). The ratio of the total number of honey bee workers collecting nectar and pollen from the flowers of *Malva parviflora*, *Pelargonium radula*, *Sinapis alba* and *Stachy stournefortii* during the day from 8:00 to 13:00(No. between prackets refere to no. of samples)

	Plant							
	Malva parviflora		*Pelargonium radula*		*Sinapis alba*		*Stachys tournefortii*	
Time	Pollen (%)	Nectar[1] (%)	Pollen[2] (%)	Nectar (%)	Pollen (%)	Nectar[3](%)	Pollen (%)	Nectar (%)
8.00	100 (9)	-	-	100 (166)	100 (137)	-	34.6 (8)	65.4 (15)
9.00	100 (28)	-	-	100 (233)	100 (240)	-	25.4 (11)	74.6 (34)
10.00	100 (39)	-	-	100 (324)	100 (390)	-	24.5 (18)	75.5 (57)
11.00	100 (68)	-	-	100 (579)	100 (438)	-	28.3 (27)	71.7 (70)
12.00	100 (84)	-	-	100 (622)	100 (519)	-	46.3 (40)	53.7 (47)
13.00	100 (54)	-	-	100 (563)	100 (533)	-	63.1 (59)	36.9 (34)

1,2,3 No visit recorded

DISCUSSION

Time of searching for food: The period of searching for food represents the time spent by bees on the flower and the period of their traveling from one flower to another on the same plant.The resting time may be determined by the type of flower frequented by the bee species in terms of the different shape of the corolla or the available reward of nectar and pollen. The results showed that the honey bee *Apis mellifera* recorded a longer resting time on the flowers of *Sinapisalba* (8.9 seconds) compared to the flowers of *Pelargonium radula* which recorded the least of resting time (7.2 seconds). This may be due to the fact that honeybees collect pollen from *Sinapis alba* and this takes a longer time to collect nectar, while honeybees have a longer resting time on *Stachy stournefortii* flowers, 8.6 seconds than on the flowers of *Malva parviflora* 8.4 seconds, and the finding may be due to that honey bee workers collect pollen and nectar together from the first plant and therefore stay longer on this plant compared to the second plant, which provides only the pollen. The relationship between the length of parts of the mouth and limiting the resting time, floral studies have confirmed that short-tongued bees quickly forage on short-crowned flowers faster than long-tongued bees, as Herrera showed in 1989 that there was a relationship between the length of the bee's mouth and its resting time on the flower, and he noticed that the wild bee *Anthophora quadrifasciata* has a mouth length of 11.1 mm and a resting time of 0.8 seconds compared with short-tongued species such *Apis* mellifera with length of the mouthparts (5.1 mm), and the period of resting time is 3.6 seconds and this indicates that the longer the mouths of bees the shorter of the resting time on the flower. While the bee workers recorded a longer time of travelling between the flowers of Stachy stournefortii (4.3 seconds) comparing to other plant flowers, the Sinapis alba flower recorded a mean travelling time of 2.8 seconds due to the proximity of flowers. Malva parviflora flowers recorded a longer travelling time with an average of 3.7 seconds and the Pelargonium radula plant recorded a travelling time of less than 3.5 seconds. In general, all species of bees recorded a time of travelling shorter than the time of resting on all plant flowers as a result of the arrangement of flowers and convergence within the flowering flower. resting and travelling time varies with the type of plant and the reward sponsored by the bees studied by Harder in 1986, which indicated that the length of time bees stand on the flowers at their visits depends on the type and quantity of nectar and pollen.

Behaviour of bees in searching for food: The results showed that the flowers of Stachys tournefortii were visited by the honey bees at 10 am by 75.5% of the (57) workers who gather the nectar, then this percentage decreased and the percentage of workers who collect pollen increased to 63.1% from (59) workers at 13.00 at noon while

ISSN: online 2617-2186 print 2617-2178

the percentage of workers that brings nectar decreased to 36.9% due to the gradually opening of the flora because of the high temperature and therefore a sufficient available amount of pollen. This behavior is consistent with what Bataw and Lamin (2001) pointed out that honey bee workers collect pollen from the *Rosmarinus officinalis* flowers after 10:00 am where pollen is available after this time. The bee collected only the pollen grains of *Sinapis alba* and that could be due to the undesirable nectar of this plant or to the lower nectar concentration, and this phenomenon was noticed by (Corbet & Delfosse, 1984) during their study on *Echium pgantagineum* flowers where honeybees collect pollen only when the concentration of nectar is less than 35%. As for the plant of *Pelargonium radula*, the workers collected only the nectar and we did not notice any worker collecting the pollen. The reason may be due to the low nutritional value of the pollen of this plant as well as the availability of other sources of pollen on adjacent plants, and this corresponds to what (Freitas & Sazima, 2003) found in the study of the mechanization of pollination in the violet flower *Viola*spp, which has an amount of nectar of 0.14 µl produced per 24 hours, but the results were different on the flowers of the berry plant. (Tian et al., 2004), indicated that the main pollinators, honey bees and *Bombus* spp, forage early in the morning on the flowers of *Impatient reptus* to obtain pollen during the first hours of opening the anthers, and return for the nectar when its concentration reaches 29.5% in the flowers of this plant.

REFERENCES

Bataw, A. A. and Lamin I. B. (2001) The behaviour of honey bee *Apis mellifera* L. that visit Rosmary flower *Rosmarinus officinalis* L. in Al-jabal Alakhder region. Almukhtar Journal of Science. 8: 26 – 41 (in Arabic)

Corbet, S. A. and Delfosse E. S. (1984). Honeybees and the nectar of *Echium plantagineum* L. in Southeastern Australia. Austral Ecology 9(2):125-139.

Fahn, A. and Shimony C. (2001). Nectary structure and ultrastructure of unisexual flowers of *Ecballium elaterium* (L.) A. Rich. (Cucurbitaceae) and their presumptive pollinators. Annals of Botany 87(1):27-33.

Freitas, L. and Sazima, M. (2003). Floral biology and pollination mechanisms in two *Viola* species—from nectar to pollen flowers? Annals of Botany 91(3):311-317.

Gegear, R. J. and T. M. Laverty. (2004). Effect of a colour dimorphism on the flower constancy of honey bees and bumble bees Canadian Journal of Zoology 7:587 -593 .

Giurfa, M. and Núñez, J. A. (1992). Honeybees mark with scent and reject recently visited flowers. Oecologia 89(1):113-117.

Harder, L. D. (1986). Effects of nectar concentration and flower depth on flower handling efficiency of bumble bees. Oecologia 69(2):309-315.

Herrera, C. M. (1989). Pollinator abundance, morphology, and flower visitation rate:analysis of the quantity component in a plant – pollinator system. Oecologia, 80:241 – 248.

Joshi, N. C. and Joshi, P. (2010). Foraging behaviour of *Apis spp*. on apple flowers in a subtropical environment. New York Science Journal 3(3):71-76.

MacKenzie, K. (1994). The foraging behaviour of honey bees (*Apis mellifera* L) and bumble bees (*Bombus* spp) on cranberry (*Vaccinium macrocarpon* Ait). Apidologie 25(4):375-383.

ISSN: online 2617-2186 print 2617-2178

Mattu, V. Raj, H. and Thakur, M. (2012). Foraging behavior of honeybees on apple crop and its variation with altitude in Shimla hills of western Himalaya, India International Journal of Science and Nature 3:296-301.

Pleasants, J. M. (1981). Bumblebee response to variation in nectar availability. Ecology 62(6):1648-1661.

Potts, S. G. Dafni A. and Ne'eman G (2001) Pollination of a coreflowering shrub species in Mediterranean phrygana: variation in pollinator diversity, abundance and effectiveness in response to fire.Oikos 92: 71–80.

Potts, S. G. Vulliamy B. Roberts S, O' Toole C. Dafni A. Ne'eman G. and Willmer P. G. (2004) Nectar resource diversity organises flower-visitor community structure. Entomologia Experimentalis et Applicata 113: 103–107

Riddell Pearce, F. C. Couvillon M. J. and Ratnieks F. L. (2013). Hive relocation does not adversely affect honey bee (Hymenoptera: Apidae) foraging. Psyche.13, 1- 8

Roubik D. W. (1989). Ecology and Natural History of Tropical Bees. Cambridge University Press, Cambridge, UK.

Steel, R. G. Torrie J. H. and Dickey D. (1980). Principles and Procedures of Statistics: a Biometrical Approach, New York. McGraw-Hill.

Sushil, S. Stanley J. Hedau N. and Bhatt J. (2013). Enhancing seed production of three Brassica vegetables by honey bee pollination in north-western Himalayas of India. Universal Journal of Agricultural Research 1(3):49-53.

Tan, K. Yang S. Wang Z.-W. Radloff S. E. and Oldroyd B. P. (2012). Differences in foraging and broodnest temperature in the honey bees *Apis cerana* and *A. mellifera*. Apidologie 43(6):618-623.

Tian, J. Liu K. and Hu G. (2004). Pollination ecology and pollination system of Impatiens reptans (*Balsaminaceae*) endemic to China. Annals of Botany 93(2):167-175.

Willmer, P. Bataw A. and Hughes J. (1994). The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. Ecological Entomology 19(3):271-284.

Yeboah Gyan, K. and S. R. J. Woodell. (1987). Analysis of insect pollen loads and pollination efficiency of some common insect visitors of four species of woody Rosaceae. Functional Ecology, 1: 229-274.

ISSN: online 2617-2186 print 2617-2178

سلوك البحث عن الغذاء لشغالة نحل العسل *Apis mellifera* Linn. الزائرة لأزهار بعض النباتات البرية بمنطقة الجبل الأخضر – ليبيا

علي عبد القادر بطاو* ونسرين خالد شريف

قسم علم الحيوان، كلية العلوم، جامعة عمر المختار، البيضاء – ليبيا

تاريخ الاستلام: 16 ديسمبر 2017 / تاريخ القبول :11 أبريل 2018
Doi:https://doi.org/10.54172/mjsc.v33i2.175

المستخلص : اجريت هذه الدراسة للتعرف علي شغالة نحل العسل *Api smellifera* في البحث عن غذائها خلال زيارتها لأزهار نباتات الحارة *Sinapis alba* والعطر شان *Pelargonium radula* والخبيز *Malva parviflora* وكذلك أزهار نبات *Stachy stournefortii*. وقد سجلت النتائج تفاوتاً في فترات البحث عن الغذاء بفرق معنوي واضح بين أزهار النباتات وبينت الدراسة أن الشغالة تقضي أطول فترة على أزهار نبات الحارة مقارنة بأزهار نبات العطر شان *Pelargonium radula* الذي سجلت أقل فترة بقاء، أما زمن انتقال نحل العسل من زهرة إلى أخرى فأظهرت النتائج إن هناك فرقاً معنوياً واضحاً حيث سجلت الشغالات في انتقالها بين أزهار نبات *Stachy stournefortii* فترة أطول وبمتوسط 4.3 ثانية وسجل أزهار نبات العطر شان فترة انتقال بمتوسط 3.5 ثانية، وأظهر نحل العسل *Apis mellifera* نشاطاً مغايراً بين الأزهار المختلفة في جمعه للرحيق أو حبوب اللقاح خلال ساعات النهار المختلفة.

الكلمات المفتاحية : سلوك البحث عن الغذاء، ليبيا ، *Apis mellifera*،الأزهار البرية، الجبل الاخضر.

* علي بطاو :Ali.bataw@omi.edu.ly، كلية العلوم، جامعة عمر المختار، البيضاء – ليبيا

Al-Mukhtar Journal of Sciences 33 (2): 124-130, 2018

Effect of Flame Retardants and 1% Stabilizer on Burning, Flammability Behaviour, and Thermal Decomposition Properties Via Polypropylene Material Treatment

Mastura A. Efhema
Department of Physics, Faculty of Science, Omer Al - Mukhtar University , Al-Bayda, Libya

Received: 24 November 2017 / Accepted: 1 May 2018.
Doi: https://doi.org/10.54172/mjsc.v33i2.177

Abstract: This study aimed to understand the mechanisn of combining the action of different types of flame netexdents an thermal stability and flammability of Polypropylene polymer (PP). PP Polymer was chosen to be blent in a twin screw extruder with the flame retardants (FR) and an additive, which is a 1% Stabilizer, to investigate Polypropylene's burning moderation and reduce it by studying its burning behavior and thermal decomposition properties. Flammability behaviour tests which is known as LOI were applied in this study. It was found that halogenated flame retardants have a little effect on reducing the rate of gas escape from polymer melt which affects the viscosity, as it was found that APP and FR245 in PP Polymer help in some leftovers of the polymer. All samples with flame retardants and no clay were burnt completely, and the flame spread was reduced and samples were self-extinguished except for the one containing the fire retardants only. All these results were investigated by XRD, Scanning Electron Microscopic (SEM), and Digital images of the charred samples of polymers.

Keywords: Materials treatment, Physical Sciences, Polymer science, Flammability behaviour, Polymers burning moderates and reduces.

INTRODUCTION

The present study is a part of a larger project exploring the production of a synthetic nano/micro fiber-composite fire retardants. This work concentrated on Polypropylene or polypropene (PP), which is a useful commodity polymer mainly used in clothing, furniture, floor coverings, medical, geotextiles, and automotive applications due to its low cost, lightweight, good mechanical properties, and low reactivity towards other chemicals. Polypropylene (PP) had higher values for tensile strength at break, the Polypropylene polymer degradation is occurring at high temperatures and the Polypropylene polymer (PP) Melting temperature is 174^{o} C if pp is 100% isotactic and the temperature of glass transition of pp polymer is -17^{o}C. The main advantage is that PP is an addition polymer made from the monomer propylene, causing it to be unusually resistant to many chemical solvents, acids and bases. However, being wholly aliphatic hydrocarbon structure, it burns very rapidly with a relatively smoke-free flame and without leaving any char residue (Zhu *et.al*, 2001). Lack of polar groups in the structure makes it difficult to react with reactive flame retardants. Additive flame retardants if used, are required in large amounts (> 20% w/w) to provide the required fire protection to products (Zhang *et.al,* 2005), this flame retardancy effect increases with increasing irradiation and vanishes with decreasing irradiation (Qin *et.al*, 2005). However, such high levels of additives cause polymer processing problems, in particular for their extrusion into thin films or fibers. The

***Corresponding Author:** Mastura A. Efhema,*abdoalshafie_mastura@yahoo.com* Department of Physics, Faculty of Science, Omer Al - Mukhtar University , Al-Bayda, Libya.

flame retardancy is sensitive to modification of the flame retardant, the use of synergists/adjuvants, and changes to the polymeric material. A detailed understanding facilitates the launch of tailored and targeted development (Morgan and Wilkie 2007). Flammability tests still require some amount of conventional flame retardants. It must be noted, however, that with this approach of using flame retardants as additives, the polymer content in the formulation is reduced compared to the unmodified polymer. In our earlier publications, we have demonstrated that nanoclays can be nanodispersed in polypropylene with a proper choice of compatibilizer, and the compounded polymer can be extruded into fibers (Gilman, 2007- Qin *et.al*, 2005). Nanoclays, although increasing the thermal stability of polypropylene and helping in char formation (Xie *et.al*, 2001), they do not reduce the flammability of PP fibers to a large extent (Gilman, 2007). Clay, nanoclay, and a small amount of flame retardant (5%) when added together to PP containing certain compatibilizers, the extruded fibers could be self-extinguished. In our previous work, we have only used ammonium polyphosphate (Qin *et.al*, 2005) whereas in this study, we use different phosphorus. The main aim of this work is to understand the mechanism of combining the action of different types of flame retardants on thermal stability and flammability of PP. A number of polypropylene samples containing compatibilizer, clay, stabilizer, and different flame retardants have been compounded in a twin screw extruder.

MATERIALS AND METHODS

The following materials were obtained from commercial sources: the seven different thermoplastic Polypropylene polymers (PP). Samples are tested after blended with UV-Stabilizer (Nor) and Flame Retardants.

Polymer Preparation: The 7 polypropylene samples composition (wt %) and additives were blended with 1% Stabilizer (Nor 116), and 5% FR as shown in Table 1.

For LOI and burning tests, coarse monofilaments (strands) were blended to the PP wt %, stabilizer 1 wt %, and FR 5 wt % (Table 1). However, the nanoclays which could be used (Cloisite 20A, and Southern Clay Products, USA) are montmorillonite clay modified with dimethyl, and dihydrogenated tallow quaternary ammonium chloride. This modified clay was chosen because of its nonpolar alkyl substituents. A Thermoelectron Prism Eurolab 16 twin screw extruder with a temperature profile over six heating zones between 179–190^{0}C was used for compounding. The polymer samples (diameter 1.8 ± 0.2 mm) were collected before pelletising.

Table (1): Mass percentages of various components in the formulations where Stabilizer 1% is NOR 116.

	Sample	pp %	Graft 1%	FR 5(%)
1	PP-Nor116	100	---	---
2	PP-APP 107	96	Polybond (pb)	---
3	*PP-NOR-NH*	91	Polybond	APP
4	PP FR 107	91	Polybond	[1]FR245
5	PP Amgard 107	91	Polybond	Amgrad NH
6	PP 3OB	96	Polybond	---
7	PP APP 3OB	92	---	APP

Note: [1] *FR = APP, NH, FR245, also PP-NOR-NH is* PP 107,FR372 or *FR245.*

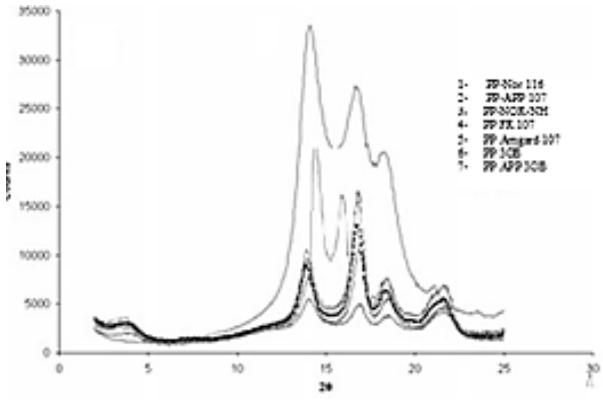


Fig. (1): The XRD curves for compounded clay-containing polymer samples showing the effect of clay and flame retardants on dispersion, where FR is APP and FR245

ISSN: online 2617-2186 print 2617-2178

Characterisation and Testing: Limiting oxygen index (LOI) (Table 2) tests were conducted using a standard procedure (Morgan and Wilkie 2007). The burning behavior of each sample was observed and noted.

Table (2): LOI and flame spread results of compounded PP polymer samples.

PP sample	LOI (%)	Burning time (sec)	Burning behaviour
1	18.4 - 8.5	2.23 -5.07	Very small flame-black ash and fast Drips.
2	17.8 - 17.9	1.46 -2.7	Very small flame, black ash.
3	17.9	4.3	Very small flame, drips, ash, and then the flame became bigger.
4	1-18.8	17.44	Very small flame, slow drips, black ash
5	17.4 - 17.5	3.73- 4.16	Very small flame.
6	18.2	35.13	Small flame, drips and then the flame became bigger
7	18.3 - 18.4	7.1- 8.3	Very small flame-black ash.

RESULTS AND DISCUSSION

The chars were examined for PP as obtained in Fig. 2 and 3, and surface characteristics and morphology by Scanning Electron Microscopy (SEM)) using a Cambridge Stereoscan 200 SEM. A Nicolet, Magna-IR Spectrometer 550 was used to study any structural changes in the chars, using KB discs containing 2% (w/w) char (Xie *et. al,* 2001, Morgan and Wilkie 2007).

PP-NOR*-FR245** **PP-NOR-*APP

Figure (2): Digital images of charred samples of heating polymers .

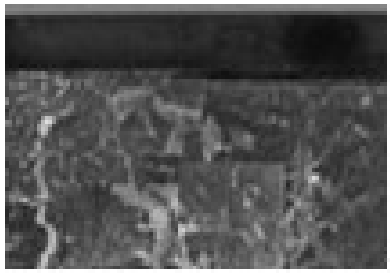


Fig (3): clay in polypropylene–clay prepared by melt compounding polypropylene, A small fraction been included.

Although the XRD study was carried out in the range 2 = 2 – 600, only the regions between 2θ = 2 – 80 were analyzed in detail. XRD curves for compounded clay-containing polymer samples showing the effect of clay and flame retardants on dispersion are presented in Figure 1. Also, the XRD and thermal analytical results of compounded polymers for the clay 1% value was 2.53nm. For sample PP-NOR, no peak could be observed, which could indicate to an exfoliation of the clay. In all flame retardant containing samples, the clay peak could be observed but at a slightly lower 2θ angle (Figure 1). Hence, higher d- spacings, and FR245 containing sample have a greater d-spacing (3.46 nm), followed by NH and Amgrad NH (sample PP Amgard 107) as recorded in Table1) (3.32, 3.31 nm), (Hu et al, 2007-B. Schartel *et. al,* 2006) APP (3.23nm) and FR245 (3.20nm).

The differences are due to the effect of different flame retardants on dispersion of the clay (Xie *et.al,* 2001, Morgan and Wilkie 2007) as seen by the microscopic studies e.g. PP- NOR-107 (Fig. 3).The transmission electron micrographs of different samples in Figure 2 showed the effect of two types of flame retardants on clay dispersion. In Figure 2a, larger particles are appeared to be the flame retardant (APP) whereas the smaller and more structured particles appeared to be clay platelets that have started to delaminate and separate, thereby indicating that delamination is taking place. This delamination is also apparent in higher resolution electron micro-

ISSN: online 2617-2186 print 2617-2178

scopic images of this sample in Figure 3. The dispersion appears to be much better for the sample PP-FR107-FR372 and PP-NOR107 (Figure 4), indicating that the flame retardant FR245 is much better dispersed compared to the nanoclay (Morgan and Wilkie 2007). In general, it can be concluded that although structures observed here are not obviously intercalated or exfoliated micro composites, a dispersion is indeed at the Nano level (Morgan and Wilkie 2007), i.e. particle size < 1μm. The clay particle widths appear to be < 0.1μm with lengths up to 0.5μm.

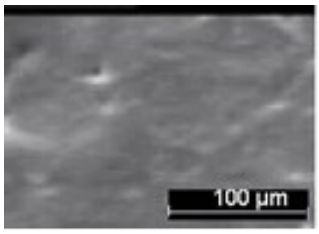


Figure (4): SEM image of charred PP-NOR-107 heated sample.

Thermal analysis: As a result, the addition of clay together with conventional flame retardants enhances both the overall thermal stability forming PP polymer. The compatibilizer helps in improving dispersion of the clay in the polymer (Morgan and Wilkie 2007), it also reduces the rate of gas escape from polymer melt affecting the viscosity. Also as can be seen from Figure 2, APP and FR245 in PP help in some char formation.

Characterization of the char residue: Selected samples are shown in Figure 2, it shows the presence of maleic-anhydride-grafted polypropylene, APP and clay in PP sample 3 (PP-NOR-NH)) has encouraged char formation (Figure 2b) whereas no char could be seen for the control sample. PP-NOR, which is still a molten polymer, has no char therefore could be observed for samples containing clays only (Fig 2b). Also as seen as in Fig. 3, the clay in polypropylene–clay was prepared by melt compounding polypropylene in a twin screw extruder. A small fraction (1–3% w/w) of modified grafted polypropylene (Morgan and Wilkie 2007) had also been included to improve dispersion of the clay (Morgan and Wilkie 2007). Scanning electron microscopic (SEM) images (Fig. 3 and 4) of these heated samples of polymers are shown in Figure (2b) and confirmed the analyses of images in Figure 2a. For example, the PP control sample in Figure (2b) showed the absence of any charred structure. The clay presence (Figure 3) (Figure 2 and 3) changed the morphology of *PP-NOR-107* polymer sample where it is seen to be more textured. Obvious char formation is not apparent until both clay and flame retardant are present together (Figure 2b), and respective char structures appear to differ albeit in an unclear way as seen in images of charred samples of polymer (Fig. 2a).

Limiting oxygen index (LOI): Limiting oxygen index (LOI) values for various samples are listed in Table 1 where it can be seen that clay and additives presence reduce them compared to either control or respective flame retardant containing samples. This behavior is not unexpected as clay presence changes the burning behavior of polymer by reducing its thermoplasticity (Beyler and Hirschler, 2002). In the presence of clay, the polymer neither melts nor lose energy via melt dripping. The polymer thus burns more easily and consistently as it was also evident from burning test results given in Table 6.

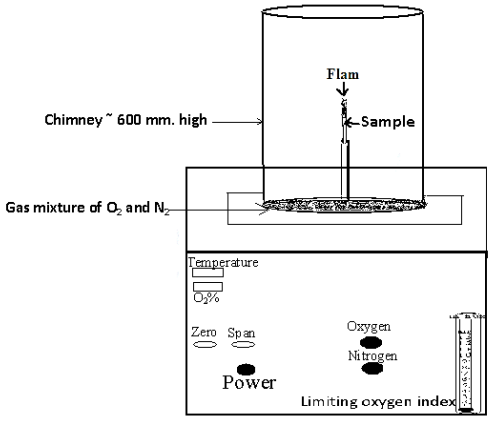


Fig. (a), The schematic of the (LO1) tests

ISSN: online 2617-2186 print 2617-2178

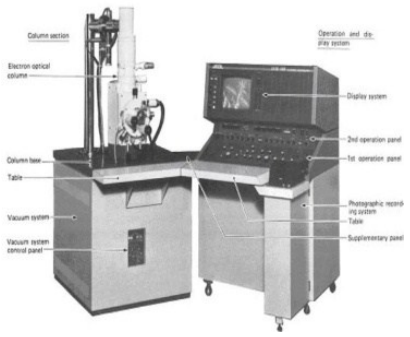


Fig. (b), Scanning electron microscopic (SEM)

Fig. (c), The XRD technique

CONCLUSION

The dispersion of nanoclay can be improved by compounding polymer-clay samples (Morgan and Wilkie 2007), and by adding a compatibilizer. LOI values are not changed significantly following the addition of clay and even in the presence of other flame retardants. However, the presence of nanoclay alone changes the thermal stability and burning behavior of the polypropylene. With additional flame retardant presence, the polymer can show self-extinguishing properties.

The effect of different flame retardant types on the thermal stability, flammability, and char formation tendency of Figure 3 optical micrographs of tape samples showed an effect of Polybond (pb) on clay dispersion. Polypropylene (PP) was studied. PP, compatibilizer, Clay, stabilizer, and different flame retardants have been compounded in a twin screw extruder to produce polymers with improved thermal and flame retardant properties. Thermal analysis has been used to study the thermal properties and LOI. All flame retardants acting in the condensed phase (phosphorus- and nitrogen- containing) (Xie *et.al*, 2001) lowered the rate of decomposition, whereas halogenated flame retardants had a little effect. Addition of 1% compatibilizer with 3% clay in sample 3 has caused microdispersion of the clay particles. However, by increasing level of compatibilizer to 3%, the dispersion at the micro level has improved as observed by the absence of clay layer. The samples compatibilizer and clay have been previously masterbatched prior to dilution and blending. Flame retardants increased the thermal stability of all samples and helped in char formation (Morgan and Wilkie 2007). Flammability behavior testes and LOI are carried out to measure flammability properties and have been examined with digital images. The decomposition significantly reduces the molecular weight, followed by an easy flowing of polymer melts. The melting wax can flow over surfaces of the specimen and drip. Thus, it is expected that the small-size dripping is independent on the cross-sectional area of the specimen, that is the first dripping time or the mass of the first drop has a little to do with the thickness of the specimen.

Outcome and future work

Up to this period, this research completed the recent developments in the area of polymer – nanocomposites science, and has suggested that the addition of just a small quantity (< 5%) of organically modified layered-silicate nanoclay (montmorillonite) (Morgan and Wilkie 2007) to a PP polymer matrix could enhance many of the properties of that polymer, including the fire performance (Beyler and Hirschler, 2002). In polypropylene (PP), the lack of polar groups in the polymer chain makes the direct intercalation or exfoliation of the nanoclays almost impossible (Beyler and Hirschler, 2002) without the use of a compatibilizer (Gilman 2007, Morgan and

ISSN: online 2617-2186 print 2617-2178

Wilkie 2007). Maleic anhydride-grafted polypropylene (PP-g-MA) can be used as a compatibilizer, which enhances the interaction between the clay and polymer with a strong hydrogen bonding between -OH or -COOH and the oxygen groups of clay (Xie *et.al*, 2001- Morgan and Wilkie 2007).

We except that the researche of Polymers science is one of the many sides and applications of material science and for the development of the Polymers and material treatments for a bright forthcoming future, for example we watched the effects of flame retardancy, FR and additives on the Polymers by assessing its burning behaviours, its chemical and physical properties, and its dripping when used to stop a fire which is now used for health and safety in real life and fire conditons.

Therefore it's very important to collect and record all the results related to these aims and more, (i.e. the flame retardancy effect increases with increasing treatment and disappears with decreasing treatment) (Schartel *et.al*, 2006), until now they found that the structures at the polycrystalline level. However, in most publications, the terms nanodispersion and nanocomposite (Morgan and Wilkie 2007) (the Nanoclay is Cloisite 20A, Southern Clay Products, USA, is a montmorillonite clay modified with dimethyl, dehydrogenated tallow-quaternary ammonium chloride (Morgan and Wilkie 2007), and this modified clay was chosen because of its non-polar alkyl substituents) are taken as mean conditions fulfilling (Morgan and Wilkie 2007).

ACKNOWLEDGEMENTS

I would like to thank the Engineering and Physical Sciences Research Council (EAPSR) and the Ministry of Defence, UK, for funding, and Rhodia Consumers Specialities Ltd and Camira Ltd. UK for their collaboration and advice.

REFERENCES

Beyler, C. L. and Hirschler, M. M. (2002). Thermal Decomposition of Polymers, The SFPE Handbook of Fire Protection Engineering,3rd Edition (Section 1, Chapter 7), National Fire Protection Association and The Society of Fire Protection Engineers, Quincy, MA, P.J. DiNenno, D.Drysdale, C.L. Beyler, and W.D. Walton, eds. pp.110-130.

Gilman, J. W. (2007). Flame retardant mechanism of polymer-clay nanocomposites. Flame retardant polymer nanocomposites:67-87.

Morgan, A.B. , and Wilkie, C.A. (2007) Flame Retardant Polymer Nanocomposites, Wiley-Interscience: New Jersey

Qin, H. Zhang, S. Zhao, C. Hu, G. and Yang, M. (2005). Flame retardant mechanism of polymer/clay nano -composites based on polypropylene. Polymer 46(19):8386-8395.

Schartel, B. Bartholmai, M. and Knoll, U. (2006). Some comments on the main fire retardancy mechanisms in polymer nanocomposites. Polymers for Advanced technologies 17 (9-10) : 772-777.

Xie, W. Gao Z. Pan W.-P. Hunter D. Singh A. and Vaia R. (2001). Thermal degradation chemistry of alkyl quaternary ammonium montmorillonite. Chemistry of Materials 13(9):2979-2990.

Zhu, J. Uhl, F. M. Morgan, A. B. and Wilkie, C. A. (2001). Studies on the mechanism by which the formation of nanocomposites enhances thermal stability. Chemistry of Materials 13 (12):4649-4654.

ISSN: online 2617-2186 print 2617-2178

تأثير مثبطات اللهب و 1% مثبت، على الاحتراق وسلوك اللهب وخصائص التحلل الحراري من خلال معالجة مادة البولي بروبيلين

مستورة افحيمة

قسم الفيزياء، كلية العلوم، جامعة عمر المختار، البيضاء–ليبيا

تاريخ الاستلام: 24 نوفمبر 2017 / تاريخ القبول: 1 مايو 2018
Doi:https://doi.org/10.54172/mjsc.v33i2.177

المستخلص: تهدف هذه الدراسة الى فهم آلية توحيد تفاعل أنواع مختلفة من مثبطات اللهب الملهجنة على استمرار التحلل الحراري من 7 عينات مختلفة من البوليمر البولي بروبيلين (PP). تم اختيار البوليمر PP لمزجه في جهاز twin screw extruder مع مثبطات اللهب (FR) والمادة المضافة وهي 1% مثبت للتحقق واختبار التحكم في احتراق البولي بروبيلين وتقليله من خلال دراسة سلوك الاحتراق وخصائص التحلل الحراري. وتم إجراء اختبارات سلوك الاشتعال والمعروفة باختبار LOIفي هذه الدراسة. كما وجد أن مثبطات اللهب المهلجنة لها تأثير ضئيل على خفض معدل ضياع وخروج الغاز الناتج عن ذوبان البوليمر والذي يؤثر على اللزوجة، كما وجد أن مثبطات اللهب APP و FR245 في بوليمر البولي بروبيلين (PP) يساعد في تشكيل بعض بقايا البوليمر. جميع العينات مع مثبطات اللهب وبدون المثبت Clay احترقت بالكامل، حجم اللهب قلَّ والعينات انطفأت تلقائيا، ماعدا العينة التي تحتوي على مثبطات اللهب فقط. كل هذه النتائج فحصت باستعمال تقنية XRD و SEMوصور دقيقة (Digital) imagesلعينات البوليمر المحترقة.

الكلمات المفتاحية: معالجة المواد، العلوم الفيزيائية، علم البوليمر، سلوك الاحتراق، التحكم وتقليل احتراق البوليمر.

مستورة افحيمة :*abdoalshafie_mastura@yahoo.com* ، كلية العلوم، جامعة عمر المختار، البيضاء – ليبيا

Al-Mukhtar Journal of Sciences 33 (2): 131-138, 2018

Prevalence of Metabolic Syndrome and Its Relation with Pro-Inflammatory Markers Among Group of Libyans

Salem M. Awami, * and Faraj Alhomry Mohamed
Faculty of medicine, Omar Al-Mukhtar University, Al-Bayda, Libya.

Received: 18 January 2018 / Accepted: 06 June 2018
Doi: https://doi.org/10.54172/mjsc.v33i2.178

Abstract: Metabolic syndrome (Met S) is known as the clustering of several metabolic abnormalities; it can be diagnosed according to several different criteria such as the International Diabetes Federation (IDF) criteria, which is intended for global application in clinical practice. Hematological tests (total Leukocyte count, differential count, platelet count and ESR) has been considered to be a marker of inflammation, several studies have examined the relationship between hematological tests, and components of metabolic syndrome; the data reveals correlations between it and metabolic syndrome in some cross-sectional studies. The study used participants recruited from Albayda and Almarj cities outpatient clinic. History of hypertension, diabetes, Blood pressure, were taken, height, weight, and waist circumference were measured body mass index (BMI, kg/m²) was calculated. Blood samples were collected for hematological tests and others. The study subject was included and categorized according to IDF criteria. A total of 192 residents aged 16 to 85 years were investigated, 80 (41.7%) participants were Males and 112 (58.3 %) were Females. The mean BMI was 29 (St. Dev±07). Rates of underweight, normal weight, overweight and obesity were 4.7%, 24.5%, 27% and 43.75%, respectively. Prevalence of Metabolic Syndrome in our study according to IDF Criteria is 25.5 % (males 12.5% and females 13%), central obesity, high plasma glucose, high blood pressure, elevated TG accounted for development of Met S. Inflammatory markers showed no significant difference between Met S and None-MetS groups, which could be explained by high prevalence of overweigh and obesity in none-MetSsyndrome persons in our study group.

Keywords: Metabolic syndrome, International Diabetes Federation (IDF), body mass index (BMI), pro-inflammatory marker.

INTRODUCTION

Metabolic syndrome (Met S) represents a cluster of health factors (like dyslipidemia, hypertension and hyperglycemia), it raises risk for heart disease and other health problems, such as diabetes and stroke (Grundy, 2008; Wilson et al., 2005).There are several definitions of Metabolic Syndrome; in 2005, the International Diabetes Federation (IDF) proposed a definition of Metabolic Syndrome intended for global application in clinical practice and represents modifications to the WHO definition and ATP III criteria (Organization, 1999). The International Diabetes Federation consensus worldwide definition of the metabolic syndrome is: Central obesity (defined as waist circumference with ethnicity-specific values) AND any two of the following:

Raised triglycerides: > 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality

Reduced HDL cholesterol: < 40 mg/dL (1.03 mmol/L) in males, < 50 mg/dL (1.29 mmol/L) in females, or specific treatment for

***Corresponding Author:** Faraj AM, *drfaraj2001@yahoo.co.uk* Faculty of medicine, Omar Al-Mukhtar University, El-Beyda, Libya

this lipid abnormality. Raised blood pressure (BP): systolic BP > 130 or diastolic BP >85 mm Hg, or treatment of previously diagnosed hypertension Raised fasting plasma glucose (FPG): >100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes If FPG is > 100 mg/dl (5.6 mmol/L), an oral glucose tolerance test is strongly recommended, but is not necessary to define presence of the syndrome.

If BMI is >30 kg/m², central obesity can be assumed and waist circumference does not need to be measured (IDF 2006). Table (1).

Table (1). Definitions and Sources of Definitions of the Metabolic Syndrome

ATP III 4	WHO 5	IDF 6
Metabolic syndrome is defined as follows: The presence of *three or more of the components listed below:	Metabolic syndrome is defined as follows: The presence of three or more of the components listed bel *Diabetes, *impaired fasting glucose, *impaired glucose tolerance, or clinically determined *insulin resistance plus at least *two of the following criteria:	Metabolic syndrome is defined as follows: *Central obesity (defined as 94 cm European men and 80 cm for Europ women) plus any *two of the following four factors:
+		
Waist circumference	Men >102 cm	Women >88 cm
Triglycerides	150 mg/dL	
HDL cholesterol	Men <40 mg/dL	Women <50 mg/dL
Blood pressure	>130/85 mmHg	
Fasting glucose	>110 mg/dL	

ATP III: Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood, Cholesterol in Adults (Adult Treatment Panel III); WHO, World Health Organization; IDF, International Diabetes Federation; HDL, high-density lipoprotein; LDL, low-density lipoprotein) Insulin resistance and/or associated hyperinsulinemia are believed to be the direct cause of the Metabolic Syndrome risk factors; Insulin resistance is enhanced by excess adipose tissue, in particular abdominal adiposity. Hyperinsulinemia may increase the production of very low-density lipoprotein triglycerides and thus raise triglycerides. Insulin resistance can raise blood pressure. Adipose tissue, once only considered to be a storage depot for triglycerides, is now recognized as a complex and active endocrine tissue that secretes many factors that regulate metabolic and vascular biology. These factors, collectively called adipokines, include adiponectin, leptin, tumor necrosis factor-alpha, resistin, angiotensinogen, interleukin-6 plasminogen activator inhibitor-1 and C-reactive protein; deregulation of these adipokines may participate in the pathogenesis of Metabolic Syndrome Chronic, sub - clinical inflammation and its association with Metabolic Syndrome is a well - documented. Inflammatory mediators have been recognized as factors that increase the risk of cardiovascular disease, but also are one cause of insulin resistance (Ross, 1999). The peripheral circulating white blood cell (WBC) count is an objective marker of acute infection, tissue damage, and other inflammatory conditions, the leukocyte count has been proposed as an emerging biomarker for predicting future cardiovascular events and mortality (Danesh et al., 1998; Imano et al., 2007). It has also been shown that macrophages residing in the adipose tissue may also be a source of pro-inflammatory markers (Boisvert et al., 1998).

The aim of the present study with a representative sample of Libyans was to investigate the prevalence of metabolic

ISSN: online 2617-2186 print 2617-2178

syndrome using the International Diabetes Federation (IDF) and assessment of the association between the pro-inflammatory markers using hematological tests (total Leukocyte count, differential count, platelet count and ESR) as possible predictors of the development of metabolic syndrome and/or its features in our population at high risk of cardiovascular disease.

MATERIALS AND METHODS

The study used participants recruited from Albayda and Almarj cities outpatient clinic, where the interview and physician examination were conducted, for each study subject, date of birth and sex, marital status, smoking, history of hypertension, diabetes and use of antihypertensive medications, antidiabetic medications. Blood pressure, height, weight, and waist circumference were measured during the physician examination. Weight and height were measured with participants standing without shoes or heavy outer garments, from which body mass index (BMI, kg/m²) was calculated. Waist circumference was measured in the erect position at the midpoint between the lowest rib and the superior border of the iliac crest. Using the World Health Organization (WHO) criteria,(Organization, 1995, 2000). BMI was categorized into four groups as underweight (BMI, <18.5), normal weight (18.5< BMI, <25.0), overweight (25.>BMI, <30.0) and obesity (BMI >30.0). Blood samples were collected for blood Hb (hemoglobin), PLT (platelet count), TLC (Total leukocyte count), DLC (differential leukocyte count), ESR (erythrocytes sedimentation rate), cholesterol, LDL (low density lipoprotein), HDL (high density lipoprotein), TG (triglyceride), urea, FPG (fasting plasma glucose), T.Bil (total bilirubin), AST (aspartate aminotransferase), ALT (alanine aminotransferase).

ALP (alkaline phosphatase). The study subject was included and categorized according to IDF (International Diabetes Federation); the exclusion criteria from the study were, active infectious disease, hepatitis, positive serology of HIV (human immunodeficiency virus), HCV (Hepatitis C Virus), and HBV (Hepatitis B Virus).

Statistical analysis: All analyses, descriptive Statistics, analysis of variance (ANOVA) and Correlations (Pearson) were done using Windows-based Minitab Statistical Package (version 11.12), and P values 0.05 were considered significant.

RESULTS

A total of 200 residents aged 16 to 85 years were investigated, 8subjects were excluded, 192 participants who had complete interview and blood sample data were included. Table (2).

Table (2). General characteristics of the study population

	Number	Mean		St Dev	MIN		Max	
Age years	192	45.56		±15.36	16 yrs		85 yrs	
		M	F		M	F	M	F
		45.65	45.49		17	16	78	85
Abd Girth cm	192	96.53		±18.89	46 cm		194 cm	
		M	F		M	F	M	F
		95.54	97.23		46	52	194	173
BMI %	192	29.06		±06.998	14.4%		53.4%	
		M	F		M	F	M	F
		27.25	30.35		14	15.10	40,40	53.40
Male	80							
Female	112							

ISSN: online 2617-2186 print 2617-2178

80 (41.7%) participants were Males and 112 (58.3%) were Females. The mean age of the participants was 45.6 (StDev. ± 15.36) years, 57.5% participants were married, 28.5% were Single, 12% were widowed and 2% were divorced, all female were non-smoker,18 % of male participants were current smokers. 50% metabolic syndrome males are smoker. The mean BMI was 29 (St. Dev±07). Rates of underweight, normal weight, overweight and obesity were 4.7%, 24.5%, 27% and 43.75%, respectively.

The mean waist circumference was 95.54 (St Dev.± 19.33) cm for male and 97.23 (St Dev. ± 18.62) cm for female (no significant difference P: 0.541). The mean systolic blood pressure and diastolic blood pressure was 134.64 (St Dev.± 27.75) mm Hg and 82.23 (StDev 18.84±) mm Hg, respectively.

The mean TG 131.01 (St Dev.± 70.4) mg%, the HDL cholesterol 50.19 (St Dev.± 15.12) mg % and fasting plasma glucose was 109.95 (St Dev.± 44.25) mg%. Table (4) shows the descriptive Statistics of Diagnostic criteria of metabolic syndrome used in the current study.

Table (3) Body mass index category and prevalence of Metabolic Syndrome

	None Met S Male	Met S Male	None Met S Femal	Met S Female	Total
Underweight 18.5	4 (2.1%)	0 (0%)	5 (2.6)	0 (0%)	9 (4.7%)
Normal >18.5 < 25	25 (13%)	2 (1%)	20 (10.4%)	0 (0%)	47 (24.5%)
Over weight >25<30	16 (8.3%)	5 (2.6%)	25 (13%)	6 (3.1%)	52(27.0%)
Obesity > 30	11 (5.7%)	17 (8.9%)	37 (19.3%)	19 (9.9%)	84(43.75)
Total	56 (29.2%)	24 (12.5%)	87(45%)	25 (13%)	192

Table (4). Descriptive Statistics of Diagnostic criteria of metabolic syndrome used in the current study

IDF Criteria	None Met S Male	Met S Male	None Met S Female	Met S Female	Total	ANOVA For None Met S & Met S
Mean Waist circumference cm	90.70	106.8	93.21	105.6	96.53	P: 0.000
Mean Triglycerides mg%	107.3	197.8	103.2	194.5	131	P: 0.000
Mean HDL cholesterol mg %	49.84	41.56	51.82	52.64	50.19	P: 0.064
Mean Fasting plasma glucose mg%	104.8	145.4	111.6	122.9	118.2	P: 0.000
Mean Systolic BP mm HG	118.6	153.6	125.04	157	134.6	P: 0.000
Mean Diastolic BP mm HG	76.29	86.50	78.30	94.50	82.23	P: 0.000

(IDF: International Diabetes Federation Criteria, Met S: metabolic syndrome, ANOVA: Analysis of Variance, BP: blood pressure) For the whole study population, the mean HB g% is 12.8, platelet count is 251000/ Cmm, Total white blood cells is 7529/ Cmm, Neutrophil % is 62 %, Lymphocyte % is 29%, bilirubin 0.50%, AST 20 U/L, ALT 20 U/L, ALP 152 U/L, while the result of different category of persons of metabolic syndrome and non-metabolic syndrome in table (5).

Table (5). inflammatory markers and other blood tests

	None-Met S		Met S		ANOVA For None Met S & Met S
Variable	Min	Max	Min	Max	P Value
Hb gm%	7.8	17.1	5.3	17.4	0.324
WBC x1000/Cmm	3.1	19.6	3.5	13.1	0.250
Neutrophil %	5.8	84	41	75	0.842
Lymphocyte %	7	88	10	50	0.405
Plateletx1000/Cmm	17	774	125	355	0.221
ESR mm./1st Hr	2	83	5	125	0.115
Bilirubin mg%	0.1	2.2	0.3	7.4	0.082
AST U/L	7	59	3	45	0.623
ALT U/L	7	124	5	44	0.813
ALP U/l	49	319	69	303	0.094
Urea mg%	9	62	13	71	0.002*

(Met S: metabolic syndrome, ANOVA: Analysis of Variance)

ISSN: online 2617-2186 print 2617-2178

DISCUSSION

prevalence of Metabolic Syndrome in our study according to international Diabetes Federation (IDF) Criteria is 25.5 % (males 12.5% and females 13%), which is low comparable to other regional and international studies; in Saudi adults is more than 28%(Aljohani, 2014), the prevalence of Metabolic Syndrome among adults in Egypt Suez Canal area is 42.1%(Maklady et al., 2014); thirty - four cross- sectional studies were analyzed with a sample of 83227, Iranian population from Jan 2005 to May 2016. With overall weighted prevalence of Met S of 31% (Dalvand *et al.* 2017); Nearly 35 percent of all U.S. adults and 50 percent of those 60 years of age or older were estimated to have the metabolic syndrome in 2011-2012,(Aguilar et al., 2015).

High plasma glucose, high blood pressure, elevated TG and increase waist circumference (reflecting central obesity) accounted for significant determinate for diagnosis of metabolic syndrome in our study population as the difference is strongly significant between metabolic syndrome and non- metabolic syndrome (table 4, P<0.001) (Carnethon et al., 2004). There is no significant difference in level of HDL cholesterol among Met S and non-MetS subjects (P 0.240) respectively. The Mean fasting plasma glucose among female study population were significantly higher than those among male (P 0.049), although the prevalence of known male diabetic in our sample study is 25% of males, 12,5 % of females; this could be explained by poor control of plasma glucose among diabetic female as the prevalence metabolic syndrome among male and female almost identical. Central obesity as an obligatory component in the IDF definition, central obesity and BMI have strong positive correlation (0.64 P 0.01). The prevalence of two components of MetS, namely central obesity and TG among male were lower than those among female, see table (3). 32.2% of female who did not satisfy the IDF criteria of metabolic syndrome, have Overweight, and Obesity Table (6); On appreciative difference of eating habits in our society according to marital status, there is no statically significant effect of marital status on prevalence of metabolic syndrome in this study; however housekeepers was identified as significant risk factors for acquiring metabolic syndrome by a cross - sectional, community-based study that covered the entire population of KSA in 2005 (Aljohani, 2014).

Table (6). prevalence of overweight and obesity in female

	None Met Female	Met S Female
Over weight >25<30	25 (13%)	6 (3.1%)
Obesity > 30	37 (19.3%)	19 (9.9%)
Total	62 (32.3%)	25(13%)

We fail to demonstrate significant increase in proinflamtory markers using hematological tests (total Leukocyte count, differential count, platelet count and ESR) in metabolic syndrome group, for example the numbers of total leukocytes, neutrophils, and lymphocytes were equal in both metabolic and non-metabolic syndrome, other inflammatory markers showed no significant difference between both groups, which could be explained by high prevalence of overweigh and obesity in non-metabolic syndrome persons in our study groups (table 5), however leukocytes increase is more evidently in acute vascular complications, and they seem to be less valuable for chronic inflammatory character, this finding was seen in few other study (Genel et al., 2014).

There significant difference of elevate blood urea level among metabolic syndrome participant (P value = 0.002); which could be linked to risk of vascular cause; of renal

ISSN: online 2617-2186 print 2617-2178

impairment due to high prevalence of atherosclerotic changes in Met S (table 5).

CONCLUSION

prevalence of Metabolic Syndrome in our study according to international Diabetes Federation (IDF) Criteria is 25.5 % (males 12.5% and females 13%), which is low comparable to other regional and international studies, There is no significant correlation between BMI and Waist circumference for diagnosis of metabolic syndrome in our population; which could be attributed to high prevalence of overweigh and obesity in non-metabolic syndrome persons in our study group 18 % of male participants were current smokers. 50% metabolic syndrome males are smoker.

Overweight, obesity, metabolic syndrome, smoking habit necessitate the work of national health system and other health financing and policy making institutions in Libya, in order to successfully diagnose and treat metabolic syndrome and create campaign to reduce obesity and smoking, trying to prevent cardiovascular complications(Grundy et al., 2005). We fail to demonstrate significant increase in proinflamtory markers using hematological tests (total Leukocyte count, differential count, platelet count and ESR) in metabolic syndrome group as possible predictors of the development of metabolic syndrome and/or its features

REFERENCES

Aguilar, M. Bhuket, T. Torres, S. Liu, B. and Wong, R. J. (2015). Prevalence of the metabolic syndrome in the United States, 2003-2012. The Journal of the American Medical Association 313 (19):1973-1974.

Aljohani, N. J. (2014). Metabolic syndrome: Risk factors among adults in Kingdom of Saudi Arabia. Journal of family & community medicine 21(3):170-175.

Boisvert, W. A. Santiago, R. Curtiss, L. K. and Terkeltaub, R. A. (1998). A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. Journal of Clinical investigation 101(2):353-363.

Carnethon, M. R. Loria, C. M. Hill, J. O. Sidney, S. Savage, P. J. and Liu, K. (2004). Risk factors for the metabolic syndrome: the Coronary Artery Risk Development in Young Adults (CARDIA) study, 1985-2001.Diabetes care 27(11):2707-2715.

Dalvand, P S. Niksima, S. H. Meshkani, R. Gheshlagh, R.G. Sadegh-Nejadi, S. Kooti, W. Parizad N. Zahednezhad H. and Afrisham, R. (2017). Prevalen - ce of Metabolic Syndrome among Iranian Population: A Systematic Review and Meta-analysis. Iranian Journal Public Health; 46(4):456–467.

Danesh, J. Collins, R. Appleby, P. and Peto, R. (1998). Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. The Journal of the American Medical Association 279(18):1477-1482.

Genel, S. Floc,a E. Kudor-Szabadi, L. M. Lucia, S. Daniel, S. and Samasca, G. (2014). The relevance of inflammatory markers in metabolic syndrome. Maedica 9(1):15.

Grundy, S. M. (2008). Metabolic syndrome pandemic. Arteriosclerosis, thrombosis, and vascular biology 28 (4):629-636.

Grundy, S. M. Cleeman, J. I. Daniels, S. R. Donato, K. A. Eckel, R. H. Franklin, B.

ISSN: online 2617-2186 print 2617-2178

A. Gordon, D. J. Krauss, R. M. Savage, P. J. Smith, S. C. Spertus, J.A. and Costa, F. (2005). Diagnosis and management of the metabolic syndrome. Circulation 112(17):2735-2752.

IDF, (2006). The International Diabetes Federation consensus worldwide definition of the metabolic syndrome. P24

Imano, H. Sato, S. Kitamura, A. Kiyama, M. Ohira, T. Shimamoto, T. and Iso, H. (2007). Leukocyte count is an independent predictor for risk of acute myocardial infarction in middle-aged Japanese men. Atherosclerosis 195 (1):147-152.

Maklady, F. A. Kamal, H. M. El-Eraky, A. Z. and Hassouna, O. H. (2014). Prevalence of metabolic syndrome among adults in Suez Canal area. The Egyptian Heart Journal 66 (1-Supplement) P 35.

WHO (1995). Physical Status: The Use and Interpretations of Anthropometry. World Health Organization Technical Report Series 854 P426.

WHO (1999). Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus.P59.

WHO (2000). Obesity: preventing and managing the global epidemic. World World Health Organization Technical Report Series 894, P252.

Sur G. Floca, E. Kudor-Szabadi, L. Sur, M. Sur, D. and Samasca, G. (2014).The Relevance of Inflammatory Markers in Metabolic Syndrome. Medica, A journal Of Clinical Medicine. 9(1): 15-18.

Ross, R. (1999). Atherosclerosis—an inflammatory disease. New England Journal Of Medicine 340(2):115-126.

Wilson, P. W. D'Agostino, R. B. Parise, H. Sullivan, L. and Meigs, J. B. (2005). Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation 112 (20) :3066-3072.

ISSN: online 2617-2186 print 2617-2178

مدي انتشار المتلازمة الأيضية وعلاقتها بدلالات سابقات الالتهابات لدي مجموعة من الليبيين

سالم محمد العوامي وفرج الحمري محمد*

قسم أمراض الباطنية، كلية الطب البشري، جامعة عمر المختار، البيضاء – ليبيا

تاريخ الاستلام: 18 يناير 2018 / تاريخ القبول: 06يونيو 2018
Doi:https://doi.org/10.54172/mjsc.v33i2.178

المستخلص: المتلازمة الأيضية هي تجمع لعديد من التشوهات الأيضية ويمكن تشخيصها وفقا لعدة معايير مختلفة مثل معايير الاتحاد الدولي للسكري ويهدف تعريف الاتحاد الدولي للسكري إلى تطبيقه وانتشاره عالميا في مجال الممارسة السريرية. وقد اعتبرت الاختبارات الخاصة والتحاليل لصورة الدم (العدد الكلي لكرات الدم البيضاء والعدد التفاضلي وعدد الصفائح الدموية، ومعامل سرعه الترسيب) مؤشرات دالة على الالتهاب وكشفت البيانات عن الارتباطات بين هذه الاختبارات والمتلازمة الأيضية في عدة دراسات عرضية هذه الدراسة استخدم فيها مشاركين من مدينتي البيضاء والمرج والذين ترددوا علي العيادات الخارجية، تتبعنا الإجراءات الطبية المتبعة من أخذ التاريخ المرضي لأمراض ارتفاع ضغط الدم والسكري والكشف السريري (قياس معدل ضغط الدم والوزن والطول ومحيط الخصر) ومن ثم حساب دليل كتلة الجسم (كجم / م 2) وجمع عينات الدم من أجل الاختبارات المختلفة. أجريت هذه الدراسة طبقا للمعايير الخاصة للاتحاد العالمي للسكري. المتلازمة الأيضية تمثل عدة عوامل مرضية مثل: ارتفاع مستوي الدهون وارتفاع ضغط الدم وارتفاع مستوي السكر في الدم والتي جميعها تزيد من معدل الخطورة لعدة أمراض منها مرض السكري ومرض ارتفاع ضغط الدم، والسكتات الدماغية والقلبية. عدد الحالات التي خضعت للدراسة 192حالة، تتراوح أعمارهم بين 16 و85 عاما. وكان 80 (41.7٪) من المشاركين من الذكور و112 (58.3٪) من الإناث. وكان متوسط مؤشر كتلة الجسم 29% (انحراف معياري ± 07). وبلغت معدلات النحافة والوزن الطبيعي وزيادة الوزن والبدانة4.7%،24.5%،27%،43.75%على التوالي. معدل انتشار المتلازمة الأيضية في هذه الدراسة حسب معيار الاتحاد العالمي للسكري كان 25.5٪ (الذكور 12.5٪ والإناث 13٪). السمنة المركزية، وارتفاع جلوكوز البلازما، وارتفاع ضغط الدم، مسؤولة عن نشوء الإصابة بالمتلازمة الأيضية. لا يوجد فارق بين علامات ودلالات الالتهابات في مجموعة المتلازمة الأيضية ومجموعة السمنة العادية وذلك لزيادة المعدل في الوزن الزائد والسمنة في أشخاص الدراسة الذين لا يعانون من المتلازمة الأيضية.

الكلمات المفتاحية: المتلازمة الأيضية، الاتحاد العالمي للسكري، دليل كتلة الجسم، سابقات علامات الالتهابات.

*فرج الحمري محمد:drfaraj2001@yahoo.co.uk ، كلية الطب البشري، جامعة عمر المختار، البيضاء – ليبيا.

Al-Mukhtar Journal of Sciences 33 (2): 139-148, 2018

Influence of Soil pH on *Azotobacter* Population With Using Microbiological Characteristics as Bio-Measurement in Arable Lands of Tripoli. N. W. Libya

Merfat T. Ben Mahmud*, Eman A. Ferjani

Department of Soil and Water, Faculty of Agriculture, Tripoli University, Tripoli -Libya

Received: 5 February 2018/ Accepted: 8 May 2018
Doi: https://doi.org/10.54172/mjsc.v33i2.180

Abstract: In the present study, 15 samples of soil were collected to isolate *Azotobacter* from the rhizosphere in different regions of Tripoli. LG specified medium was used for the isolation of bacteria and were purified on the same medium for identification and characterisation.The colonies were identified through microscopical and biochemical tests and the results obtained were classified as *Azotobacter* sp. Subsequently, the microbial population was calculated by colony count method. The soil pH, total nitrogen content (N), total phosphorus content (P) and organic carbon (OC) in soil were determined. The results of this study indicated to effects positive and negative of soil pH levels on *Azotobacter* population. In the estimation of above chemical properties of all soil samples it was showed that bacterial population differs significantly among the different soil samples.

Keywords: *Azotobacter* population, Soil properties**.**

INTRODUCTION

The genus *Azotobacter* has a great attention to stimulate plant-growth-promoting rhizobacteria (PGPR), and their role in rising the growth and health of plants. Moreover, many other species have the ability to produce compounds with antimicrobial activity. The genus *Azotobacter* was discovered by Martinus Beijerinck in 1901. *Azotobacter* belongs to the phylum proteobacteria, class: Gammaproteobacter order: pseudomonadales, family Azotobacteraceae, comprises more species among them: *Azotobacter vinelandii*, *A. chroococcum*, *A. salinestris*, *A. nigricans*, *A. beijerinckii*, *A. paspali*, and *A. armeniacus* (Kennedy *et al*. 2005). *Azotobacter* is an aerobic free living diazotrophic bacteria generally distributed in different soils. *Azotobacter* play an important role in the nitrogen cycle in nature. In addition, the bacteria are the most significant genera found in rhizosphere gramineae (Dart & Day, 1975). The plant growth is improved, both directly through nitrogen fixation, excretion of growth promoting and producing plant growth substances such as indole-3-acetic acid (IAA), increasing solubilization of mineral phosphates and indirectly through producing hydrogen cyanide, siderophore and antifungal antibiotics by means of the bacteria (Benizri et al., 2001). Several studies mentioned nitrogen fixation, production of phytohormones, vitamins and increasing of food uptake as the reasons for yield increase of inoculated maize with *Azotobacter* (Gonzalez - Lopez et al., 1991). *Azotobacter* inoculation with oak seedlings results in positive growth responses was suggested by (Pandey et al., 1986). Moreover the inoculation of barley grains with *Azotobacter* in leads to growth of plant length, dry matter, soil nitrogen content in sand and nitrogen deficient lands (Shehata et al., 2005). *Azotobacter* can produce antifungal antibiotics which inhibit *Rhizoctonia solani* growth (Zarrin *et al*. 2009), *Azotobac-*

***Corresponding Author:** Merfat T. Ben Mahmud, dr.mbenmahmoud@yahoo.com Faculty of Agriculture, Tripoli University, Tripoli -Libya

ter is found in many environments such as soil, water, surfaces of roots (rhizosphere) and leaves (phyllosphere). Also, some species appear in the tropical and polar regions. Their frequency is different in various soils. They are frequent in neutral to alkaline soils and rarely found in acidic soils (Jensen & Petersen, 1955) *Azotobacter* is gram–negative, nitrogen–fixing soil bacteria that have extremely high respiration rates. *Azotobacter* can fix at least 10 mg nitrogen per gram of carbohydrate (Becking, 1992).

This bacterium is an obligate aerobic. Nitrogen fixation is achieved by the enzyme nitrogenous, which reduces $N_2$to NH_3. However, this enzyme is extremely sensitive to oxygen in *Azotobacter* species. High respiration rates and conformational protection of the enzyme are suggested as two factors which make nitrogen fixation possible in an aerobic environment (Hill and Sawers, 2000) Reduction of O_2 by *Azotobacter* species occur at such a high rate that large amounts of superoxide radicals are produced (Vikhe, 2014). *Azotobacter* is a free-living fixing bacteria and related to soil organic components, and the amount of nitrogen fixation is lower in *Azotobacter* compared to the associative and symbiotic bacteria as reported by (Hammad, 1998).The ecological distribution of *Azotobacter* is a complex subject and related to a variety of factors which determine the presence or absence of this bacterium in soil. It has been demonstrated soil properties and climate conditions are two most important factors that affect the distribution of this microorganism (Dobereiner & Pedrosa, 1987). These characteristics include organic matter content, moisture, pH and C/N ratio (Gonzalez - Lopez et al., 1991).

Different studies showed that some *Azotobacter* mutants can fix N_2 in the presence of excess NH_4^+ which is related to *Azotobacter* industrial applications(Terzaghi, 1980). The mutants are of industrial significance, because they hinder mobilization in alginate beads and provide the opportunity to produce ammonia (which can be used as plant fertilizer). So *Azotobacter* is used in biofertilizer and biotechnological processes (Tejera et al., 2005).

As well as, this study aimed to address the effect of the chemical properties of different soil samples in different regions of Tripoli as soil pH, total nitrogen content (N), total phosphorus content(P) and organic carbon (OC) on *Azotobacter* population.

MATERIALS AND METHODS

Collection of Soil samples : This experiment was conducted in Soil microbiology laboratory at Faculty of Agriculture, University of Tripoli at the end of Winter season of 2016, Fifteen soil samples were collected from the different cultivated and uncultivated regions in Tripoli area. 1 kg of soil was collected randomly from the rooting zone at a depth of (5- 30 cm) below the surface with three replicates of each of soil samples. Prior to commencement of the experiment, bulk soil samples were air-dried, cleaned and passed through a 5 mm sieve to determine particles chemical analysis.

Measuring of Soil chemical properties

microbiological properties: The chemical properties of soil mean most chemical interactions with or between minerals in soil environment. Such as soil ph, Cation Exchange Capacity, Basic Saturation…ect. While microbiological properties of soil belong biological activity in soil, such as N-fixation, humus formation. Which include microorganisms activity in soil environment. The pH of soil was measured using pH meter. Organic carbon was observed by using the method of (Walky & Black, 1934) and Seeley and Vandemark (1981). The estimation of total nitrogen was done by using the Kjeldahl method and the total phosphorus content (P) was analysed using Olsen method by extracting soil samples with 0.5M $NaHCO_3$ (pH 8.5)

ISSN: online 2617-2186 print 2617-2178

at a solid to solution ratio 1:20 for 30 min (Olsen, 1954) and using Spectrophotometer at 660nm wavelength (Table1).

Table (1). Chemical and microbiological properties of soil samples

Soil samples	*Azotobacter population (1gram soil x 10.0000)*	pH	Total N%	Total P%	OC%
1	4.98	7.3	5.87	7.96	2.14
2	6.5	7.1	57.16	11.9	3.63
3	4.37	7.3	33.53	22.88	1.88
4	1.17	8.4	5.23	23.4	1.63
5	2.84	8	8.25	20.96	1.52
6	1.13	8.2	1.35	14.9	2.68
7	5.33	7.8	34.75	19.3	2.34
8	5.7	7.23	33.08	14.19	2.79
9	3.8	7.5	27.58	19.85	1.42
10	5.65	7.5	44.62	15.66	3.22
11	5.63	7.4	47.04	19.90	0.76
12	6.13	7.2	59.75	23.2	4.7
13	5.49	7.17	37.98	5.70	2.79
14	4.23	7.4	32.58	22.89	3.08
15	1.95	8.1	3.8	12.56	1.736

Isolation of *Azotobacter:* The soil paste–plate method of (Becking, 1981) was used to Isolate of *Azotobacter* from soil samples. Each soil sample was mixed thoroughly with approximately 0.5 g of mannitol, 0.5 g of $CaCO_3$, 0.12 ml of 10% aqueous K_2HPO_4 solution, 0.12 ml of 10% aqueous $MgSO_4$ solution, and some extra distilled water was also added in order to obtain a soil paste, and then incubated at 30°C for 48h. Then brown, glistening, slimy *Azotobacter* colonies were grown on the soil surface. Subsequently, brown blots of soil paste surface were placed on Jensen medium and purified (Subba Rao, 1993). Bacterial colonies were transferred to plates of the same medium.

Identification of bacteria: Isolates were cultured on plates of N-free LG medium for identification and characterization. In gain isolates from each soil samples were Gram-stained using standard procedures. Morphology characterization was determined using a compound microscope in oil immersion (1000 x) about 100 colonies were chosen at random at all the colonies from the rhizosphere of soil samples whatever their size, shape and color were transferred onto other plate to check for purity. All the colonies grown on the plates were about 1mm diameter and white with flat margins initially glossy and gummy but turned into glistening colonies with clear slime upon further growth (Brenner *et al.* 2004). The following biochemical tests were used: catalase, oxidase, nitrate reduction and movement (Seeley and Vandemark 1981). Moreover, the carbon sources utilization test was determined by using the phenol red medium and dispensed into sterile test tubes. Then, 0.5% (w/v) of the glucose, fructose, malonate, mannitol, caproate, inositol, malonate, rhamnose and starch were separately added to 24 h old inoculated culture and incubated at 30°C for 24 h. Temperature is perhaps the most important environmental factor determining the activity of microorganisms in soil. The effect of temperature on the growth rate was determined by patching the bacteria on to the LG medium and incubated at different tempera-

ISSN: online 2617-2186 print 2617-2178

tures 15, 18, 21, 32, 37°C. The growth of bacteria colonies until 5 days after the incubation indicated their ability to grow in the cited temperatures. Motility was assessed using a Craigie tube with a semi-solid medium Nitrate reduction was tested by inoculating trypticase-nitrate tubes with the colonies and then incubating at 27°C for 48 h. One ml of sulfanilic acid was added to each tube, and then 1 ml of dimethyl 1-naphthylamine solution (Seeley and Vandemark (1981). Some of the pure isolates from each soil samples were defined by direct use of microscopic morphological characteristics and compared to some of the known and available cultures and then were characterized using the criteria of (Brenner *et al.* 2004).

Estimation of *Azotobacter* population:To estimate numbers of *Azotobacter* in each soil sample the colony count method was used (Cappuccino and Sherman 1987). Ten grams of soil sample was transferred into the 250 ml of the conical flask containing 90 ml of sterilized distilled water and was shaken for 30 min at 150 rpm, and 1 ml of this solution was added to the test tubes containing 9 ml sterilized distilled water to prepare 10^{-2} dilution. The latter solution was mixed and one ml of this solution was transferred to another test tube containing 9 ml sterilized distilled water to prepare 10^{-3} dilution again and the same method was followed to prepare 10^{-5} dilution. Subsequently, 0.1 ml each of the dilutions was transferred to a plate containing Jensen medium and was dispensed to the above medium equally. Three replicates were maintained for each sample. 50 mg cycloheximide was added to medium as fungal growth inhibitor. The plates were incubated at 30°C for 3-7 days and *Azotobacter*–like colonies were counted. The dilutions with colony number between 10 – 60 colonies were accepted. The average colony number was calculated in the three replicates multiplied in ten and the reverse of appropriate dilution.

Statistical Analysis: The data were subjected to correlation analysis of variance using statistical program (SPSS software) Table (2). The differences among various treatment means were compared using Tukey's family error test (standard deviation) at a probability of P = 0.05.

RESULTS AND DISCUSSION

Isolation of *Azotobacter:*The pure isolates of bacterial colonies were sub cultured from the 60 isolates on the LG medium for further studies. The colonies formed by these bacteria on the LG medium were small, transparent, circular, flat, and slimy with regular border (Fig 1).

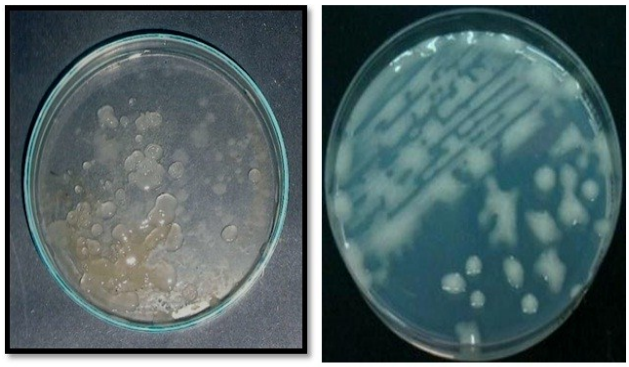


Fig (1). Colonies of *Azotobacter* on LG medium Incubated at 30°C for 3-7 days

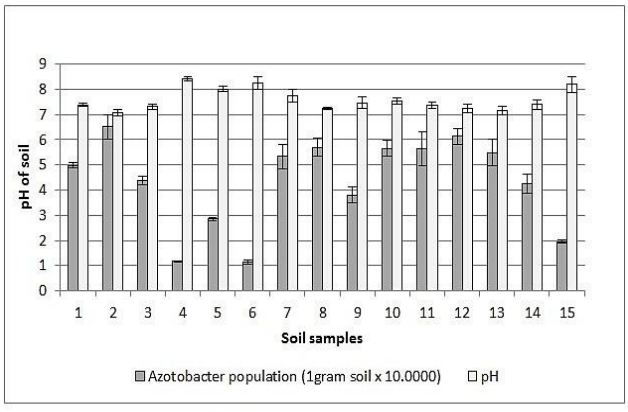
Bacteria were Gram-negative with rounded ends. Also, the isolates produced yellow-green and brown pigments and were put in one group. Biochemical and morphological characteristics of these bacteria included the following: motile, catalase positive, oxidase activity positive and Nitrate reduction positive. The utilization of glucose, fructose, malonate, mannitol, caproate, inositol, malonate, rhamnose but not starch was detected. Bacteria grew well in LG medium with 15, 18, 21, 32, 37°C temperatures. On the basis of cultural, morphological and biochemical characteristics a total of 15 soil isolates were classified according to (Brenner *et al.* 2004) as *Azotobacter* sp. It is in agreement with the obtained results by (Ahmad et al., 2008).

Relationship of chemical properties of soil

ISSN: online 2617-2186 print 2617-2178

with *Azotobacter* population:

Soil pH : The soil pH are definition as the negative logarithm of the hydrogen ion concentration pH = -log (Bashan).The soils are referred to as being acidic, neutral or alkaline, depending on their PH values, also these categories of soils are dividing to group of classes according to degrees of acidity of soil. Among these classes soil neutral is 6.5-7.5 PH, and soil slightly alkaline is 7.5-8.0 PH, while soil moderately alkaline is 8-8.5 PH. In this study the *Azotobacter* population was determined in different 15 soil samples. The result showed that all samples contained *Azotobacter* and the high population of *Azotobacter* was observed in soil samples with the range of pH 7 - 7.5. whereas *Azotobacter* population relatively continue in range PH of soil slightly alkaline, while *Azotobacter* population was decline as soon as commence at a zone of moderately alkaline soil as in fig.(2). Also observed through soil samples 4,5,6 and 15 from table (1) and fig.(2) decrease in the amount of total N% in soil with decrease in the *Azotobacter* population while happen increasing in soil alkaline levels, on other hand the opposite was happen in soil samples 2 and 12. This explain an existence increasing relationship between *Azotobacter* population and total N% in neutral soils.



Error bars represent the standard deviation ± SD

Fig (2). The relationships between soil pH with *Azotobacter* population Several studies indicated that the soil pH value influences the *Azotobacter* population (Jensen & Petersen, 1955).

The studies showed that all soils with pH of above 7.2 (pH range 7.3 - 8.5) contained *Azotobacter* and, in the pH ranges of 7.0 - 7.4, 6.5 - 6.9, and 6.0 - 6.4, the percentage of *Azotobacter* was 90, 58, and 35%, respectively (Gonzalez - Lopez et al., 1991; Kanungo et al., 1997) has indicated that the optimum pH for the growth of *Azotobacter* sp. is near to 7. Also, (Becking, 1981) noted that *Azotobacter* population in tropical soils with pH of above 7.5 differs between 10^2 and 10^4 per gram of soil. Various studies proved the linear relationship between soil bacterial communities and pH value. Then, other studies showed bacterial population in the range of pH 4-8 and observed that increasing pH value and bacterial population are interrelated (Rousk et al., 2010).

Total Nitrogen (N): Nitrogen is a major limiting nutrient for crop production, in case absence of a source of nitrogen compound, plant need to organisms for fixed atmospheric nitrogen. from table (1) notice, increasing of nitrogen percentage in soil which was correspond to increasing of *Azotobacter* population in soil. this mean there is relationship between *Azotobacter* growth and nitrogen fixation in soil fig.(3). however this relationship was limiting with soil PH levels, although major soil PH values for soil samples which examined were situated between neutral to moderately alkaline soils, nevertheless *Azotobacter* appearing tend to growth in neutral soils more than slightly alkaline soils. whereas *Azotobacter* growth recorded fast retreat in moderately alkaline soils. table(1). (Bashan, 1990) reported that, the *Azotobacter* population is low in dry and temperate zones like America and Mexico. The total nitrogen contents were suggested as the factors influencing the microbial population (Ahmed *et al*. 2008).

ISSN: online 2617-2186 print 2617-2178

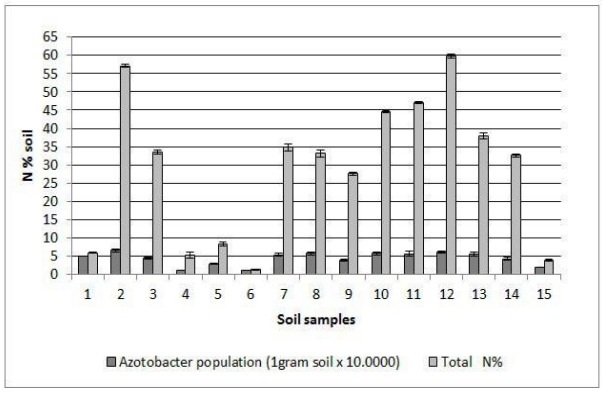


Fig (3). The relationships between total Nitrogen (N%) with *Azotobacter* population

Total phosphorus (P): In this study, the soil samples which had neutral PH such as 2,12,and 13 in table(1) appearing various values of total phosphorus percentage in soil with *Azotobacter* population, whereas soil samples of moderately alkaline soil 4,5,6 and 15 table(1). Showing increasing in total P%. as opposite to *Azotobacter* population fig.(4). But these changes in total P% do not explain the decrease in *Azotobacter* growing in alkaline soil, because organic phosphorus decrease quickly with soil depth such as soil organic matter. Secondly the source of P in soil. in case of the source of available P in soil Ca phosphates the level of soil PH will changes from neutral to high alkaline, while in case Al and Fe phosphates are predominates P mineral in soil with PH levels below 6.5. Therefore, the value of soil PH above 8 was probably responsible for the decrease of *Azotobacter* population in soils of region of study. Some studies reported that, the native soil P is mostly unavailable to plants because its low solubility, therefore the P solubilizing bacteria and *Azotobacter* sp can play an important role in improving P bioavailability in soil, on the other side the population of rhizobacteria which includes *Azotobacter* had a different influence on phosphorus in soil (Wu et al., 2005). phosphorus is also a major nutrient for microorganisms and suggested to be the factors influencing the microbial population.

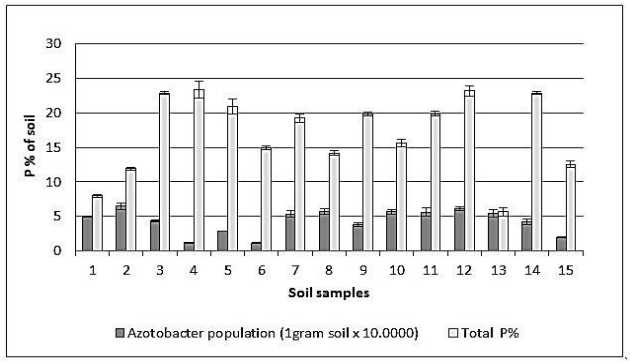


Fig (4). The relationships between total phosphorus (P%) with *Azotobacter* population

Organic Carbon (OC) : The organic carbon in soil are an importance indicator for existence soil organic matter. Through soil samples which were contain high percentage of O.C as,2,10, 12 and 14 in table (1) and fig. (5). Also, observed at same time increasing in the *Azotobacter* population and total N percentage at neutral soil 7-7.5 PH. On other hand, soil samples which were contain low O.C such as 11,9,5 and 4 do not appearing any response to *Azotobacter* population, particularly soil samples (6 ,11) which showing a clear disagreement in their contain of O.C and *Azotobacter* population. So, that mean do not there any direct relationship between O.C % and *Azotobacter* population at soils of region of study.

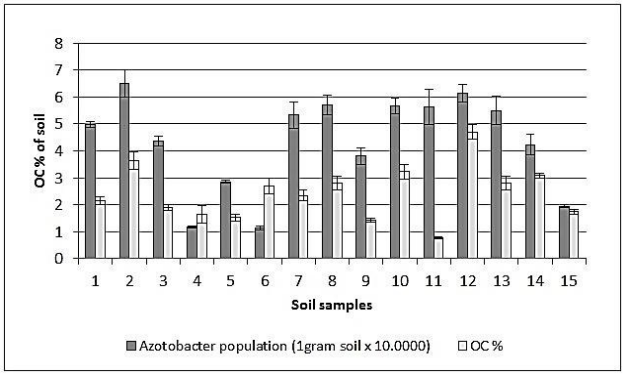


Fig (5). The relationships between Organic carbon (OC %) with *Azotobacter* population

A range of environmental factors like pH, organic carbon, total N and total P determine and influence the distribution of soil microbi-

ISSN: online 2617-2186 print 2617-2178

al population (Kennedy & Smith, 1995). Organic carbon is one of the main factors influencing the number, composition and activities of microbial population(Wardle, 1992). Lalfakzuala *et al*. (2008) found that gramineae influenced soil microbial number and soil respiration positively. Organic carbon affects both the chemical and physical properties of the soil (Channal et al., 1989). Properties influenced by organic matter include: soil structure, diversity and activity of soil organism, which might be beneficial and harmful to crop production. Soil organic matter is an accumulation of dead plant matter and animal residues (Campbell et al., 2000). Furthermore, The findings from this study showed that there was a Linear relationship ($p<0.01$) was observed in different soil samples for bacterial population as shown in Table (2) and significant relationship between soil pH, total N, total P and organic carbon with microbial population, so that the number of bacterial population per gram of soil increased by increasing the compounds which, indicated, there is a significant relationship between the soil organic and mineral matters on the microbial population (Coutinho *et al*. 1999).

Table (2). Relationship with soil pH, total nitrogen, total phosphorus and organic carbon between bacterial population.

Variables	Coefficient Correlation (r)
pH	0.93**
Total N	0.95**
Total P	0.90**
OC	0.75**

CONCLUSION

In conclusion, this study has shown that a significant correlation between soil pH, total nitrogen, total phosphorus and organic carbon the chemical properties of different soil samples from different soil regions of Tripoli-Libya on *Azotobacter* population which had a greater influence on it.

REFERENCES

Ahmad, F. Ahmad, I. and Khan, M. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research 163(2):173-181.

Bashan, Y. (1990). Short exposure to Azospirillum brasilense Cd inoculation enhanced proton efflux of intact wheat roots. Canadian Journal of Microbiology 36(6):419-425.

Becking, J.-H. (1981). The family Azotobacteraceae. Pages 795-817 The prokaryotes. Springer.

Becking, J. H. (1992). The family Azotobacteraceae. Pages 3144-3170 The Prokaryotes. Springer.

Benizri, E. Baudoin, E. and Guckert, A. (2001). Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Science and Technology 11(5):557-574.

Campbell, C. Zentner, R. Selles, F. Biederbeck, V. McConkey, B. Blomert, B. and Jefferson, P. (2000). Quantifying short-term effects of crop rotations on soil organic carbon in southwestern Saskatchewan. Canadian Journal of Soil Science 80(1):193-202.

Channal, H. Alagawadi, A. Bharamagoudar, T. Udupa, S. Patil P. and Mannikeri, I. (1989). *Azotobacter* population as influenced by soil properties in some soils of North Karnataka. Current science. Bangalore 58(2):70-71.

Dart, P. and Day, J. (1975). Nitrogen fixation in the field other than by nodules. Soil Microbiology. Butter Worth Sci. Publication, London.

ISSN: online 2617-2186 print 2617-2178

Dobereiner, J. and Pedrosa, F. O. (1987). Nitrogen-fixing bacteria in nonleguminous crop plants. Science Tech Publishers.

Gonzalez-Lopez, J. Martinez-Toledo, M. Reina, S. and Salmeron, V. (1991). Root exudates of maize and production of auxins, gibberellins, cytokinins, amino acids and vitamins by *Azotobacter* chroococcum in chemically-defined media and dialysed-soil media. Toxicological & Environmental Chemistry 33(1-2):69-78.

Hammad, A. (1998). Evaluation of alginate-encapsulated *Azotobacter* chroococcum as a phage-resistant and an effective inoculum. Journal of Basic Microbiology 38(1):9-16.

Jensen, V. and Petersen, E. (1955). Taxonomic studies on *Azotobacter chroococcum* Beij. and *Azotobacter beijerinckii* Lipman. Royal Vet. Agric. Coll. Copenhagen Yearbook:107-128.

Kanungo, P. Ramakrishnan, B. and Rao, V. R. (1997). Placement effects of organic sources on nitrogenase activity and nitrogen-fixing bacteria in flooded rice soils. Biology and Fertility of Soils 25(2):103-108.

Kennedy, C. Rudnick, P. MacDonald, M. and Melton, T. (2005) Genus III: *Azotobacter*. In: Garrity GM, editor. Bergey's Manual of Systematic Bacteriology. The Proteobacteria, Part B, the Gammaproteobacteria. 2nd edition. Vol. 2. New York, USA: Springer. 384- 402.

Kennedy, A. C. and Smith, K. (1995). Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil 170(1):75-86.

Olsen, S. R. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department Of Agriculture; Washington.

Pandey, R. Bahl, R. and Rao, P. (1986). Growth stimulating effects of nitrogen fixing bacteria (biofertiliser) on oak seedlings. Indian Forester 112(1):75-79.

Rousk, J. Bååth, E. Brookes, P. C. Lauber, C. L. Lozupone, C. Caporaso, J. G. Knight, R. and Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4(10):1340.

Shehata, S. Saleh, S. and Junge, H. (2005). Response of sexual expression and productivity of squash plants to some biofertilizer treatments. Egypt Journal Applied Science 20(12B):680-690.

Tejera, N. Lluch, C. Martinez-Toledo, M. and Gonzalez-Lopez, J. (2005). Isolation and characterization of Azotobacter and Azospirillum strains from the sugarcane rhizosphere. Plant and Soil 270(1):223-232.

Terzaghi, B. E. (1980). Ultraviolet sensitivity and mutagenesis of Azotobacter. Microbiology 118(1):271-273.

Vikhe, P. (2014). *Azotobacter* species as a natural plant hormone synthesizer. Research Journal of Recent Sciences 3:59-63.

Walky, A. and Black, I. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid in soil analysis. 1. Experimental. Soil Sciences 79:459-465.

ISSN: online 2617-2186 print 2617-2178

Wardle, D. (1992). A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews 67(3):321-358.

Wu, S. Cao, Z. Li, Z. Cheung, K. and Wong, M. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125(1-2):155-166.

ISSN: online 2617-2186 print 2617-2178

دراسة مدى تأثير حموضة التربة pH على مستعمرات بكتيريا *Azotobacter* باستخدام الخصائص المكروبيولوجية كمقياس حيوي في الأراضي الزراعية بمنطقة طرابلس شمال غرب ليبيا

ميرفت الطاهر بن محمود، إيمان علي الفرجاني

كلية الزراعة، قسم التربة والمياه – جامعة طرابلس، طرابلس – ليبيا.

تاريخ الاستلام: 5 فبراير 2018 / تاريخ القبول :8 مايو 2018
Doi:https://doi.org/10.54172/mjsc.v33i2.180

المستخلص : جنس *Azotobacter* لديه القدرة على تحفيز – تعزيز نمو النبات (PGPR)، ودورها في رفع ونمو وصحة النباتات. وعلاوة على ذلك، فالعديد من الأنواع الأخرى لديها القدرة على إنتاج مركبات منها المركبات المضادة لنشاط الميكروبات. في هذه الدراسة جمعت 15 عينة من منطقة الجذور لتربة مزروعة في مناطق مختلفة من طرابلس شمال غرب ليبيا لتحديد درجة تفاعل التربة pH، ونسبة النيتروجين الكلي (N)، الفوسفور (P) والكربون العضوي (OC) في التربة وأيضا لعزل بكتيريا *Azotobacter* و استخدمت البيئة الغذائية LG لعزل البكتيريا وتنقيتها على نفس البيئة لوصفها و تعريفها وقد تم تحديد جنس البكتيريا من خلال الفحص المجهري والاختبارات البيوكيميائية للعينات، وأظهرت النتائج أن البكتيريا المتحصل عليها تابعة لبكتيريا *Azotobacter* وفقا لدليل Bergey's Manual of Systematic Bacteriology (2004) وقد تم حساب أعدادها عن طريق العد للمستعمرات البكتيرية لتحديد مدي تأثير الخواص الكيميائية للترب على هذه المستعمرات. نستنتج من نتائج هذه الدراسة أن المستعمرات البكتيرية لبكتيريا *Azotobacter* تتأثر إيجابياً وسلبياً وفقا لمستويات حموضة التربة pH.

الكلمات المفتاحية : بكتيريا *Azotobacter* ، خواص التربة ، أعداد البكتيريا.

*ميرفت الطاهر بن محمود: dr.mbenmahmoud@yahoo.com، كلية الزراعة، جامعة طرابلس، طرابلس – ليبيا.

Al-Mukhtar Journal of Sciences 33 (2): 149-160, 2018

Effect of Wastewater on Heavy Metal Accumulation in *Cystoseria sp.* (Brown algae) and *Enteromorpha. sp*. (Green algae) in Derna Coast, Libya

Masoud.M.M.Zatout[1] , Yousef K. A. Abdalhafid[2], Salmeen. H.Alhage[2]

[1]*Zatout, Alhayat Organization for Wildlife & Marine Protection, Derna, Libya*
[2] *Department of Botany , Faculty of Sciences, Omar Al-Mukhtar University, Al-Bayda Libya*

Received: 8 December 2017/ Accepted18 April 2018
Doi: https://doi.org/10.54172/mjsc.v33i2.179

Abstract: In this work was studied effect of wastewater pollution on heavy metal accumulation in *Cystoseria sp.*, (brown seaweed) and *Enteromorpha sp.* (Green algae), in an effort to gain some insight into the level of metal contamination which might exist in the coastal marine environment along the Derna coast. Assessed by measuring the concentration of heavy metals as Pb, Zn, Mn and Cd, in the algae tissue and seawater. The results indicate concentrations of metals were invariably slightly higher in *Enteromorpha sp*., than in *Cystoseria sp.,* at all sampling stations. The metals concentrations recorded for the different tissues and sites of the present study confirm the higher concentrations usually observed in summer. The average MPI was highest (0.86) for the both species inhabiting S6 station and least (0.29) at S1 station. In general, the all of heavy metals show no detrimental effects on the domestic aquatic environment of Derna coast. However, must be monitored continuously to ensure that they stay at harmless levels.

Keywords: pollution, heavy metal, bio-indicators, wastewater, macroalgae.

INTRODUCTION

The wastewater discharge consists primarily of untreated human wastes and domestic wastes. Heavy metals are common types of toxic substance present in sewage sludge that affect aquatic life. The outfall has been in operation for over many years and discharges into the Derna coast area. Wastewater is any water that has been adversely affected in quality by anthropogenic influence. Wastewater can originate from a combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water, and from sewer inflow or infiltration(Beijer & Jornelo, 1979). The rationale for using organisms to assess levels of contamination in the marine environment, and the criteria by which these organisms are selected as suitable biomonitors are well documented (Al-Homaidan et al., 2011; Alkhalifa et al., 2012; Dokulil, 2003; Khaled et al., 2014; Kureishy et al., 1995; Li & Huang, 2012). The value of algae as bio-indicators has already been recognized in the mid of 19th century (Wu et al., 2014). Seagrass or macroalgae can be used as biomonitors to give information on concentrations of heavy metal or changes in metal availabilities in the surrounding environment, besides their abundance in various environmental systems(Campanella et al., 2001; Capiomont et al., 2000). In general, algae are widely distributed in the aquatic environment and are sedentary, easy to collect, identify, and the bioaccumulation of trace metals occur in high degrees; satisfying all the fundamental requirements for bioindicators (Campanella et al., 2001). Brown algae species can accumulate high concentrations of metals in contaminated ecosystems, and as a result, they are chosen as heavy metal biomonitors in coastal areas(Amado-Filho et al.,

***Corresponding Author:** Masoud.M.M.Zatout, marwan2004h@yahoo.co.uk Zatout, Alhayat Organization For Wildlife & Marine Protection, Derna, Libya

1997; Andrade et al., 2010; Astorga-España et al., 2008; Karez et al., 1994; Villares et al., 2002). Green algae are considered an indicator of contamination (Brown et al., 1999). These algae were chosen to conduct the investigation as bio-indicators for heavy metals pollution of Derna city coastal area at the east of Libya. Due to their they were almost available in all sites during field study. Due to their short life cycle, algae respond quickly to environmental changes and are thus a valuable indicator of water pollution(Domingues & Galvão, 2007). Many of the chemical tested are basic parts of components of domestic and sewage sludge. The nature of any potentially toxic substances depends mainly on the types of wastes entering the sewage system. Heavy metals are common types of toxic substance present in sewage sludge that affect aquatic life.Libya is located in lies the Mediterranean Sea, it is ranked 124th out of 142 countries on an Environmental Sustainability Index, which places the country well down the list signifying a country with serious environmental degradation (WEF, 2002). This region is very important, due to it having distinct environmental characteristics associated. Also it has an environment similar to other regions in Southern Europe such as Italy, the Greek islands and Turkey(Azzawam, 1984). The study area is located along the Derna coast, in Jabal Akhdar region the north east part of Libya at latitude 32° North and 22° East. This area which are exposed to different degrees of pollution. Marine pollution in Derna coastal, due to the enormous increment of sewage discharged into the aquatic environment, has drawn the attention in the last decades, see figure 2. Little quantitative data are available on the concentration of metals in seaweeds general pollution of this area. The main aim of this work was to investigate the effect of wastewater on heavy metal accumulation in *Cystoseria sp.,* (Brown algae) and *Enteromorpha sp.,* (Green algae), which might exist in the coastal marine environment along the Derna beaches.

MATERIALS AND METHODS

Study Area : The Derna city is a part of the Jabal Akhdar, were is situated in North east of Libya. Climatically, the study area of the Derna coast is influenced by the Mediterranean Sea to the north. The rainfall is erratic in quantity, frequency and distribution. It attracts considerably more reliable rainfall than other coastal regions of Libya between autumn to early spring, with the mean annual rainfall ranges between 450 and 650 mm, 24-30 % falling in January. The temperature is 8-13°C in winter and 22-27°C in summer Winds are northern in winter but southern and eastern southern in other seasons. Six stations were chosen including the Derna Harbor (S1), Shahat company (S2), Post station (S3), Republic station (S4), Algarod station (S5) and Desalination station (S6), see figure 1.

Fig. (1).Locations of the six study sites, which are located along the Derna coast. The inset map shows the location of the study area in Libya

ISSN: online 2617-2186 print 2617-2178

Fig. (2). Marine pollution in Derna coastal, due to the sewage discharged into the aquatic environment

Measurements: The concentrations of metals were measured in macroalgae species in triplicates in two seasons at six coastal stations along the Derna city coast. Seawater samples were also collected to detect their metal contents in order to gain more information on the environmental conditions of the area and possible bioaccumulation patterns, were cleaned with distilled water and all algal samples were air dried at 90°C (Al-Homaidan et al., 2011), were collected during two seasons in spring and summer, 2016. The two collected algae were identified as *Cystoseria sp.,* (Brown algae) and *Enteromorpha sp.,* (Green algae) and then kept to analyze for heavy metals (manganese (Mn), zinc (Zn), cadmium (Cd), and lead (Pb). The concentrations of manganese, zinc, cadmium and lead were determined in the collected algal samples according to previously reported methods (Al-Homaidan et al., 2011). 500 mg (dry weight) of each algal dried sample were placed in acid washed digestion tubes. 25 ml of concentrated nitric acid was added to each tube and the contents were evaporated to about dry ness. After cooling, 20 ml of double deionized water was added to each tube and the contents were filtered through 0.45 µm millipore filters. The solutions were then transferred to 25 ml acid washed volumetric flasks and the volumes were completed to 25 ml with double distilled deionized water. All samples were analyzed in triplicates and the concentrations were expressed in µg (metal) per gram dry weight (alga) and the mean values were recorded (Al-Homaidan et al., 2011). All analysis was made in three replicates. Statistical analysis was based on SPSS (Version 11.0) program (Al-Homaidan et al., 2011).

RESULT AND DISCUSSION

Heavy Metals in Algae: The present data revealed of *Cystoseria sp.*, recorded a relatively high (Pb) concentration in summer at the S6 station 1.50±0.3 (µg/g), while recorded their lowest values in both summer and spring seasons at the S3, S5 stations with 0.78±0.02 and 0.40±0.08 (µg/g) respectively, table 1. The *Cystoseria sp.,* recorded a rela-

ISSN: online 2617-2186 print 2617-2178

tively high (Mn) concentration in summer and spring at the S1 station with 1.74±0.1 and 1.16±0.02 (µg/g) dry weight), while recorded their lowest values in summer and spring at the S2 station 0.72±0.02 and 0.36±0.02 (µg/g) respectively. The *Cystoseria sp.,* collected from the S6 station in both summer and spring seasons recorded the highest concentration of (Zn) level 1.15±0.06 and 0.81±0.01 (µg/g), respectively. Whereas the lowest levels were found at S3 station 0.69±0.03 (µg/g) in summer, followed by at S1 station 0.27±0.007 (µg/g) in spring, see figures 3 and 4. This results are in good agreement with those found by (Strezov & Nonova, 2003) who reported concentrations of (Pb) level between in two *Cystoseira sp*., in most stations from the Bulgarian Black Sea coast. On the other hand, the *Cystoseira sp.,* in the present study, recorded lower values of (Pb) compared than that reported by many authors as; (Al-Masri et al., 2003; Schintu et al., 2010) for algae collected from the south-western Sardinia, Italy; (Khaled et al., 2014) for *Cystoseira sp.,* in Marsa-Matrouh beaches, Egyptian in Mediterranean Sea; (Strezov & Nonova, 2007). Average while, the present data revealed a lower values of (Mn) than that recorded by (Strezov & Nonova, 2007). To compare the present data with those previously studied by many authors revealed that *Cystoseira sp.,* recorded lower values for (Zn) compared than that reported by many authors as; (Akcali & Kucuksezgin, 2011; Al-Masri et al., 2003; Culha et al., 2013; Khaled et al., 2014; Schintu et al., 2010; Strezov & Nonova, 2007)in Kastamonu station in Black Sea. In the present study recorded lower values of (Cd) compared than that reported by many authors as; (Akcali & Kucuksezgin, 2011; Al-Masri et al., 2003; Khaled et al., 2014; Schintu et al., 2010; Strezov & Nonova, 2007). On the other hand, the value of average concentration for (Pb) was in this study, higher to those found by (Culha et al., 2013) for *Cystoseira sp.,* in marine algae samples of all sampling stations in Black Sea, Marmara Sea and Mediterranean Sea. Also by (Sawidis et al., 2001) for *Cystoseira sp.,* in the Aegean Sea, Greece. As well, the value of average concentration for (Zn) was in this study, higher than to those found by (Culha et al., 2013) for *Cystoseira sp.,* in marine algae sample of samsun station in Black Sea. While, the value of average concentration for (Cd) was in this study, comparable to those found by (Culha et al., 2013) for *Cystoseira sp.,* in marine algae samples of all sampling stations in Black Sea, Marmara Sea and Mediterranean Sea. The present data revealed of *Enteromorpha sp.,* recorded a relatively high Pb concentration in summer and spring seasons at the S6 station 1.92±0.3 and 1.63±0.03 (µg/g) respectively.

Table (1). Average concentrations (µg g^{-1} dry weight) with standard error of means in *Cystoseira sp.,* collecting during spring and summer seasons 2016, along stations of Derna coast.

Stations	Metals								Ave.
	Pb		Mn		Zn		Cd		
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	
S1	0.61±0.01	1.20±0.03	1.16±0.02	1.74±0.1	0.27±0.007	0.75±0.008	0.007±0.01	0.007±0.01	0.72
S2	0.41±0.02	0.85±0.03	0.36±0.02	0.72±0.02	0.41±0.02	0.71±0.04	ND	ND	0.58
S3	0.60±0.05	0.78±0.02	0.58±0.03	0.93±0.02	0.59±0.06	0.69±0.03	0.001±0.002	0.001±0.001	0.52
S4	0.69±0.1	0.96±0.03	0.59±0.06	0.81±0.03	0.77±0.01	0.93±0.03	ND	ND	0.79
S5	0.40±0.08	0.84±0.03	0.57±0.03	0.89±0.03	0..68±0.03	0.86±0.01	ND	ND	0.71
S6	1.13±0.03	1.50±0.3	0.95±0.09	1.12±0.09	0.81±0.01	1.15±0.06	ND	ND	1.11
Ave.	0.831		0.868		0.718		0.004		

ISSN: online 2617-2186 print 2617-2178

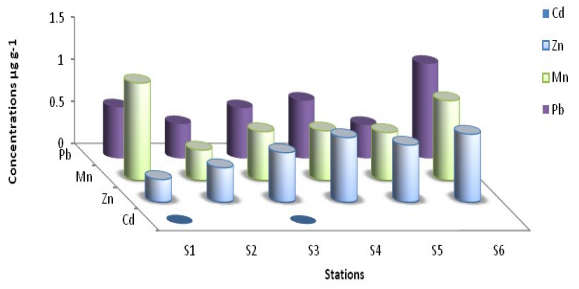


Fig (3) . Average concentrations (µg g^{-1} dry weight) in *Cystoseira sp.,* collecting during spring season 2016, along stations of Derna coast.

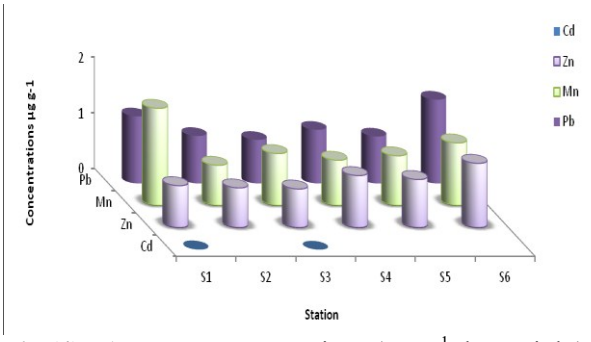


Fig (4) . Average concentrations (µg g^{-1} dry weight) in *Cystoseira sp.,* collecting during summer season 2016, along stations of Derna coast.

while recorded their lowest values in summer and spring at the S4 0.96±0.03 and 0.69±0.1 (µg/g) respectively, table 2. While, *Enteromorpha sp.,* recorded a relatively high Mn concentration in summer and spring at the S4 station 1.91±0.03 and 1.85±0.06 (µg/g), while recorded their lowest values in summer and spring at the S3 station 1.12±0.02 and 0.88±0.03 (µg/g) respectively. In *Enteromorpha sp.,* we also have data for Zn, which was at a maximum at S6 station, 1.82±0.06 µg/g in summer. Minimum levels of Zn in this alga 0.05±0.01 (µg/g) in spring, were found at S4 station, see figures 5 and 6.Comparison of the present data with those previously studied by many authors revealed that the concentrations of Zn, Pb and Cd for *Enteromorpha sp.,* lower than that recorded by (Schintu et al., 2010) along the south-western Sardinia, Italy; (Khaled et al., 2014) in the Marsa-Matrouh beaches in Mediterranean Sea; (Strezov & Nonova, 2007) for *Enteromorpha sp*. The present data revealed a lower values of Mn and Zn for the *E. compressa* than that recorded by (Villares et al., 2001), also for Zn with those previously studied by many authors as(Culha et al., 2013; Say et al., 1990). While, the value of average concentration for (Pb) was in this study, higher to those found by (Culha et al., 2013) in Black Sea, Marmara, Sea and Mediterranean Sea; (Sawidis et al., 2001) in the Aegean Sea, Greece. The value of average concentration for (Zn) was in this study, higher than to those found by (Culha et al., 2013) for *Enteromorpha sp*., in marine algae in Ordu station in Black Sea. As well, the value of average concentration for (Cd) was in this study, comparable to those found by (Culha et al., 2013) for *Enteromorpha sp*. in marine algae samples of all sampling stations in Black Sea, Marmara Sea and Mediterranean Sea. Many authors reported that the expected levels of Zn in *Enteromorpha sp*., are in the range 10-50 µg g^{-1} and 95-130 µg g^{-1}, for uncontaminated and contaminated sites respectively (Phillips, 1990; Stenner & Nickless, 1975).

Table (2). Average concentrations (µg g^{-1} dry weight) with standard error of means in *Enteromorpha sp.,* collecting during spring and summer seasons 2016, along stations of Derna coast.

Stations	Metals								Ave.
	Pb		Mn		Zn		Cd		
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	
S1	0.86±0.01	1.20±0.03	1.22±0.02	1.72±0.1	1.74±0.007	1.25±0.008	0.001±0.01	0.003±0.01	1.00
S2	0.95±0.02	1.26±0.03	1.21±0.02	1.35±0.02	0.20±0.02	0.39±0.04	0.06±0.01	0.04± 0.00	0.68
S3	1.08±0.05	1.55±0.02	0.88±0.03	1.12±0.02	0.60±0.06	0.88±0.03	0.05±0.002	0.006±0.001	0.77
S4	0.69±0.1	0.96±0.03	1.85±0.06	1.91±0.03	0.05±0.01	0.19±0.03	0.002±0.0	0.006±0.001	0.71
S5	1.13±0.08	1.55±0.03	0.92±0.03	1.60±0.03	1.07±0.03	1.14±0.01	0.004±0.0	0.007±0.02	0.93
S6	1.63±0.03	1.92±0.3	1.24±0.09	1.56±0.09	1.53±0.01	1.82±0.06	0.007±0.0	0.007±0.0	1.21
Ave.	1.23		1.38		0.91		0.02		

ISSN: online 2617-2186 print 2617-2178

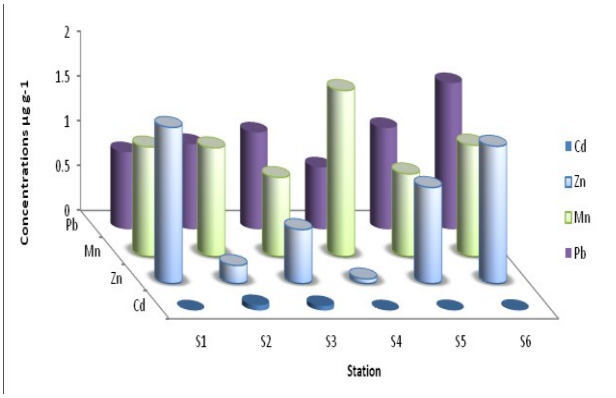


Fig (5) . Average concentrations (µg g^{-1} dry weight) in *Enteromorpha sp.,* collecting during spring season 2016, along stations of Derna coast.

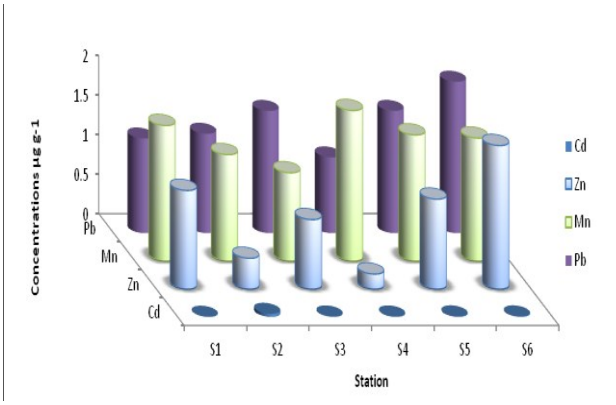


Fig (6) . Average concentrations in *Enteromorpha sp.,* collecting during summer season 2016, along stations of Derna coast.

Heavy Metals in Water: Determination of heavy metal concentrations in marine algae samples is usually preferred in the seawater and sediment samples. Heavy metal concentrations in seawater are very low and show wide fluctuation. At the same time, heavy metal levels in the sediment samples can be changed by organic matter content, grain size composition, pH and oxidation-reduction potential, etc. (Farías et al., 2002; Förstner, 1985). In our study, low metal contamination was found in S1 for Pb, Mn, Cd and Zn in seawater compared with contamination metals accumulated in the tissues of algae. The concentration of the metals in the six sampling sites followed the order of Mn>Zn>Pb>Cd. The average of heavy metals concentrations in sea water were lower at site S2 and relatively higher at site S1 (Table 3, Figures 7 and 8).

Mn was the most abundant metal, whereas Cd was the least abundant. The other metals exhibited intermediate concentrations and variability among the six sites. Largely, metals contents in seawater, from S1 obtained in this work are intermediple, site S1 in our study were the most contaminated for Pb in summer and spring seasons. This site is seem heavily affected by human activities and was the closest to the wharf. Pb concentrations from seawater in this study ranged between 0.12 and 0.54 (µg l^{-1}), were this result agree with (Chakraborty et al., 2014) for water sea along the coast of the Gulf of Kutch in India. While site S4 were the most contaminated for Mn in both summer and spring seasons, between 0.24 and 0.52 (µg l^{-1}). As well, the maximum values of Cd metal were also determined at site S5 in both summer and spring seasons between 0.003-0.05 (µg l^{-1}). These were much similar results obtained by (El-Adl et al., 2017), along Al-Hanyaa coastline, Libya, as well as the standard limits of Environmental Protection (EPA, 2014).

In addition, results in this study show that the site S6 were the most contaminated for Zn in summer and spring seasons. Despite of the discharge of wastes in sea water, our results were much lower than those reported from along Al-Hanyaa Coastline, Libya 5.4–7.4 (µg l^{-1}) (El-Adl et al., 2017).

ISSN: online 2617-2186 print 2617-2178

Table (3). Average heavy metals concentrations (µg l^{-1}) with standard error of means in the selected marine water collecting during spring and summer seasons 2016, along stations of Derna coast.

Stations	Metals								Ave.
	Pb		Mn		Zn		Cd		
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	
S1	0.47±0.01	0.54±0.05	0.37±0.03	0.42±0.08	0.17±0.01	0.21±0.008	ND	0.002±0.0	0.31
S2	0.15±0.08	0.20±0.05	0.24±0.03	0.31±0.01	0.11±0.2	0.17±0.05	ND	0.001±0.0	0.20
S3	0.16±0.08	0.21±0.05	0.36±0.01	0.41±0.08	0.28±0.2	0.35±0.05	ND	0.002±0.0	0.22
S4	0.12±0.01	0.20±0.06	0.41±0.01	0.52±0.01	0.29±0.05	0.31±0.01	ND	0.004± 20.0	0.23
S5	0.37±0.01	0.41±0.01	0.36±0.02	0.45±0.08	0.28±0.01	0.36±0.01	ND	0.005± 20.0	0.29
S6	0.12±0.01	0.21±0.08	0.28±0.01	0.31±0.08	0.41±0.01	0.52±0.01	0.002±0.0	0.004±0.01	0.23
Ave.	0.23	0.30	0.34	0.40	0.26	0.32	0.00	0.00	
	0.26		0.37		0.29		0.00		

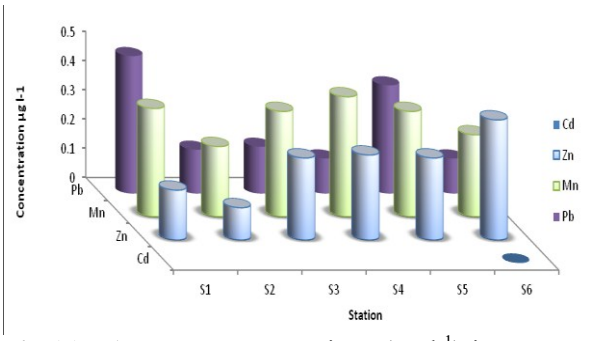


Fig (7) . Average concentrations (µg l^{-1}) in water collecting during spring season 2016, along stations of Derna coast.

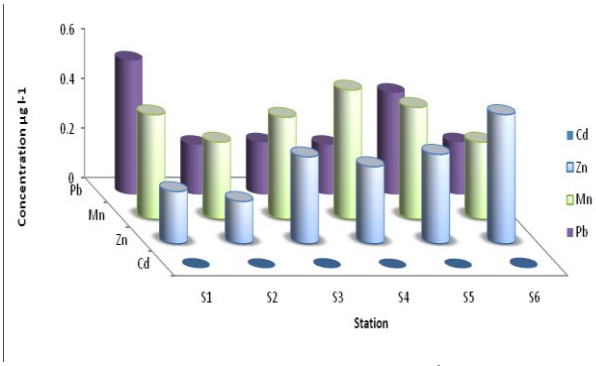


Fig (8) . Average concentrations (µg l^{-1}) in water collecting during summer season 2016, along stations of Derna coast.

Metal Pollution Index (MPI): The average MPI was highest for the algae species inhabiting S3 and S6 stations, intermediate for the algae species of S4 and S5 stations, and least for the algae species of S1 and S2 stations (Table 4 and Fig. 9 and 10). Metal pollution index (MPI) can be used to compare the average heavy metal content of different algal species within the same site or among different sites (El-Adl et al., 2017).The ability to accumulate heavy metals was highest in *Chaetomorpha sp.,* which was substantially higher than *Enteromorpha sp.,* at most stations. This points to a marked genotypic variability in heavy metal accumulation and agrees with the findings of (Khan et al., 2015) who reported that some macroalgae can concentrate heavy metals in their tissues to several times higher than those in the ambient water.

Table (4). Metal Pollution Index (MPI) of algae species along six stations of Derna coast

Algae	S1	S2	S3	S4	S5	S6	Average
Enteromorpha sp.	0.32	0.37	0.37	0.16	0.31	0.41	0.32
Chaetomorpha sp.	0.32	0.36	1.09	1.21	1.06	1.16	0.87
Average	0.32	0.37	0.73	0.69	0.69	0.79	

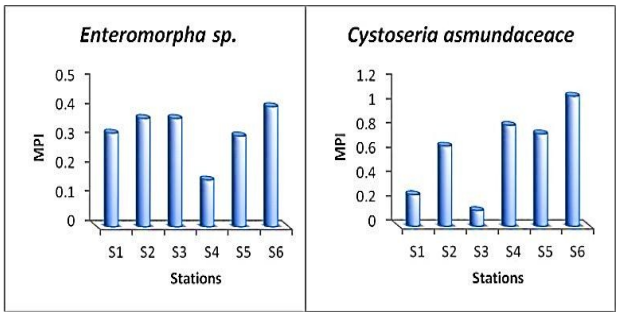


Fig (9) . Metal pollution index (MPI) of *Enteromorpha sp.,* and *Chaetomorpha sp.*, algae along the six stations of Derna coast.

ISSN: online 2617-2186 print 2617-2178

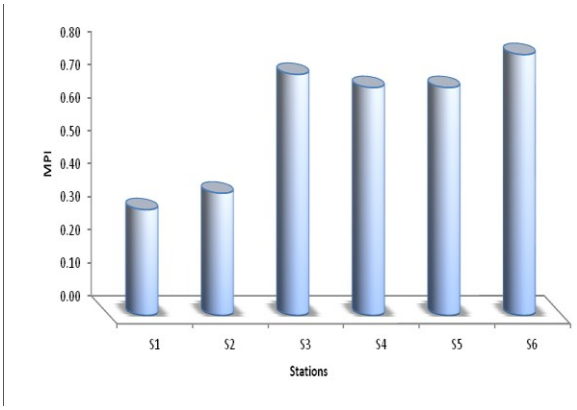


Fig (10). Metal pollution index (MPI) of algae along the six stations of Derna coast.

Heavy Metals in Two Seasons: The metals concentrations recorded for the different tissues and sites of the present study confirm the occurrence of significant seasonal variability, with maximum concentrations usually observed in summer. Such seasonal variability in trace metal concentrations have been reported by other authors for Pb and Cu in Posidonia oceanica (Malea et al., 1994), for Cd, Cu, Pb and Zn in Posidonia australis (Ward, 1987), and for Cd, Cu, Pb and Zn in Zostera marina (Lyngby et al., 1982). The latter authors found that maximum concentrations of heavy metals were recorded when the growth has ceased, and decline of these metals occurred at the beginning of the growth season. In this study, the concentrations of zinc, lead and manganese in algae varied seasonally, the concentrations collected in summer show exhibiting significantly higher metals levels than those of individuals collected during the spring. Generally, as previously reported by many authors as(Akcali & Kucuksezgin, 2011; Brown et al., 1999); there may be a number of reasons for the seasonal differences found, including: environmental factors, such as variations in metal concentrations in solution, interactions between metals and other elements, salinity, pH etc., metabolic factors, such as dilution of metal contents due to growth; or they may be due to interactions between both kinds of factors, different genetic capacities for metals concentration.

CONCLUSION

The obtained heavy metal contents indicated that different species demonstrated various degrees of metal accumulation. High levels of Mn were detected in the both species of algae. Drainage of waste into the coast of city are probably the main cause of this problem. The information compiled from the above recommendations can then direct strategies for initial environmental surveys to investigate contamination of heavy metals in Derna coast.

REFERENCES

Akcali, I., and Kucuksezgin F. (2011). A biomonitoring study: heavy metals in macroalgae from eastern Aegean coastal areas. Marine pollution bulletin 62(3):637-645.

Al-Homaidan, A. A., Al-Ghanayem A. A., and Alkhalifa A. H. (2011). Green algae as bioindicators of heavy metal pollution in Wadi Hanifah Stream, Riyadh, Saudi Arabia. International Journal of Water Resources and Arid Environments 1(1):10-15.

Al-Masri, M., Mamish S., and Budier Y. (2003). Radionuclides and trace metals in eastern Mediterranean Sea algae. Journal of Environmental Radioactivity 67 (2):157-168.

Alkhalifa, A. H., Al-Homaidan A. A., Shehata A. I., Al-Khamis H. H., Al-Ghanayem A. A., and Ibrahim A. S. (2012). Brown macroalgae as bio-indicators for heavy metals pollution of Al-Jubail coastal area of Saudi Arabia. African Journal of Biotechnology 11(92):15888-15895.

Amado-Filho, G., Andrade L., Reis R., Bastos W., and Pfeiffer W. 1997. Heavy metal concentrations in seaweed species from the Abrolhos reef region, Brazil. Pages

ISSN: online 2617-2186 print 2617-2178

1843-1846 *in* Proc. 8th Int. Coral Reef Symp., Panamá. 2: 1843-1846

Andrade, L. R., Leal R. N., Noseda M., Duarte M. E. R., Pereira M. S., Mourão P. A., Farina M., and Amado Filho G. M. (2010). Brown algae overproduce cell wall polysaccharides as a protection mechanism against the heavy metal toxicity. Marine pollution bulletin 60(9):1482-1488.

Astorga-España, M. S., Calisto-Ulloa N. C., and Guerrero S. (2008). Baseline concentrations of trace metals in macroalgae from the Strait of Magellan, Chile. Bulletin of environmental contamination and toxicology 80(2):97-101.

Azzawam, S. (1984). Al Jabal Al Akhdar: A Natural Geography Study. Garyounis University. Benghazi, Libya.

Beijer, K., and Jornelo A. (1979). Sources, transport and transformation of metals in the environment In: Friberg L, Nordberg GR and Vouks VB (Eds.). Handbook on the toxicology of metals. Elsevier/North Holland Biomedical Press, New York. pp. 47-63.

Brown, M., Hodgkinson W., and Hurd C. (1999). Spatial and temporal variations in the copper and zinc concentrations of two green seaweeds from Otago Harbour, New Zealand. Marine Environmental Research 47(2):175-184.

Campanella, L., Conti M., Cubadda F., and Sucapane C. (2001). Trace metals in seagrass, algae and molluscs from an uncontaminated area in the Mediterranean. Environmental Pollution 111(1):117-126.

Capiomont, A., Piazzi L., and Pergent G. (2000). Seasonal variations of total mercury in foliar tissues of Posidonia oceanica. Journal of the Marine Biological Association of the United Kingdom 80(6):1119-1123.

Chakraborty, S., Bhattacharya T., Singh G., and Maity J. P. (2014). Benthic macroalgae as biological indicators of heavy metal pollution in the marine environments: A biomonitoring approach for pollution assessment. Ecotoxicology and environmental safety 100: 61-68.

Culha, S. T., Kocbas F., Gundogdu A., and Culha M. (2013). Heavy Metal Levels In Marine Algae From The Black Sea, Marmara Sea And Mediterranean Sea. Rapports et procès-verbaux des réunions - Commission internationale pour l'exploration scientifique de la mer Méditerranée, 40:828.

Dokulil, M. (2003). Algae as ecological bio-indicators. Trace metals and other contaminants in the environment 6(285-327.

Domingues, R. B., and Galvão H. (2007). Phytoplankton and environmental variability in a dam regulated temperate estuary. Hydrobiologia 586(1):117-134.

El-Adl, M. F., El-Katony T. M., and Bream A. S. (2017). Effect of sewage pollution on macroalgal diversity and heavy metal accumulation along Al-Hanyaa coastline, Libya. Advances in Environmental Biology 11(2):52-60.

EPA. 2014. National recommended water quality criteria. Washington, DC: U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology.

Farías, S., Arisnabarreta S. P., Vodopivez C., and Smichowski P. (2002). Levels of essential and potentially toxic trace metals in Antarctic macro algae.

ISSN: online 2617-2186 print 2617-2178

Spectrochimica Acta Part B: Atomic Spectroscopy 57(12):2133-2140.

Förstner, W. (1985). The reliability of block triangulation. Photogrammetric Engineering & Remote Sensing 51(8):1137-1149.

Karez, C., Magalhaes V., Pfeiffer W., and Amado Filho G. (1994). Trace metal accumulation by algae in Sepetiba Bay, Brazil. Environmental Pollution 83(3):351-356.

Khaled, A., Hessein A., Abdel-Halim A. M., and Morsy F. M. (2014). Distribution of heavy metals in seaweeds collected along Marsa-Matrouh beaches, Egyptian Mediterranean Sea. The Egyptian Journal of Aquatic Research 40(4):363-371.

Khan, M., Trivellini A., Fatma M., Masood A., Francini A., Iqbal N., Ferrante A., and Khan N. A. (2015). Role of ethylene in responses of plants to nitrogen availability. Frontiers in plant science 6:927-941.

Kureishy, T. W., Abdel-Moati M., and Al-Muftah A. (1995). Marine Algae as bioindicators of pollution levels in the Arabian Gulf. Qatar University Sciences Journal, 15 (1): 215- 221

Li, L., and Huang X. (2012). Three tropical seagrasses as potential bio-indicators to trace metals in Xincun Bay, Hainan Island, South China. Chinese Journal of Oceanology and Limnology 30(2):212-224.

Lyngby, J. E., Brix H., and Schierup H.-H. (1982). Absorption and translocation of zinc in eelgrass (Zostera marina L.). Journal of experimental marine biology and ecology 58(2-3):259-270.

Malea, P., Haritonidis S., and Kevrekidis T. (1994). Seasonal and local variations of metal concentrations in the seagrass Posidonia oceanica (L.) Delile in the Antikyra Gulf, Greece. Science of the total environment 153(3):225-235.

Phillips, D. J. 1990. Use of macroalgae and invertebrates as monitors of metal levels in estuaries and coastal waters, *In* Furness ,R. W. and Rainbow , P. S. (eds.), Heavy Metals in the Marine Environment. CRC Press, Boca Raton, Florida. p. 81–99.

Sawidis, T., Brown M., Zachariadis G., and Sratis I. (2001). Trace metal concentrations in marine macroalgae from different biotopes in the Aegean Sea. Environment International 27(1):43-47.

Say PJ, Burrows JG, Whitton BA (1990) Enteromorpha as a monitor of heavy metals in estuaries. Hydrobiologia 195:119–126

Schintu, M., Marras B., Durante L., Meloni P., and Contu A. (2010). Macroalgae and DGT as indicators of available trace metals in marine coastal waters near a lead–zinc smelter. Environmental monitoring and assessment 167(1):653-661.

Stenner, R., and Nickless G. (1975). Heavy metals in organisms of the Atlantic coast of SW Spain and Portugal. Marine pollution bulletin 6(6):89-92.

Strezov, A., and Nonova T. (2003). Monitoring of Fe, Mn, Cu, Pb and Cd levels in two brown macroalgae from the Bulgarian Black Sea coast. Intern. J. Environ. Anal. Chem. 83(12):1045-1054.

Strezov, A., and Nonova T. (2007). Marine Macroalgae For Assessment Of Radionuclide And Heavy Metal

ISSN: online 2617-2186 print 2617-2178

Pollution In The Black Sea. Rapports et procès-verbaux des réunions - Commission internationale pour l'exploration scientifique de la mer Méditerranée, 38.

Villares, R., Puente X., and Carballeira A. (2001). Ulva and Enteromorpha as indicators of heavy metal pollution. Hydrobiologia 462(1):221-232.

Villares, R., Puente X., and Carballeira A. (2002). Seasonal variation and background levels of heavy metals in two green seaweeds. Environmental Pollution 119(1):79-90.

Ward, T. (1987). Temporal variation of metals in the seagrass Posidonia australis and its potential as a sentinel accumulator near a lead smelter. Marine Biology 95(2):315-321.

World Economic Forum (2002). Environmental Sustainability Index. Main Report p 301.

Wu, N., Schmalz B., and Fohrer N. (2014). Study Progress in Riverine Phytoplankton and its Use as Bio-Indicator a Review. Austin Journal Hydrology 1(1):1-9.

ISSN: online 2617-2186 print 2617-2178

تأثير المياه العادمة على تراكم المعادن الثقيلة في الطحالب البنية (*Cystoseria sp*) و الطحالب الخضراء (*Enteromorpha sp.*) في ساحل درنة، ليبيا

مسعود زعطوط[1]، يوسف عبدالحفيظ [2] وسليمان الحاجي[2]

[1] منظمة زعطوط لحماية الحياة البرية والبحرية في درنة، درنة، ليبيا

[2] قسم النبات، كلية العلوم، جامعة عمر المختار، البيضاء، ليبيا

تاريخ الاستلام: 15 مايو 2017 / تاريخ القبول: 4 أغسطس 2018

Doi:https://doi.org/10.54172/mjsc.v33i2.179

المستخلص : في هذا العمل تم دراسة تأثير تلوث المياه العادمة على تراكم المعادن الثقيلة في *Cystoseria sp.*, (الطحالب البنية) و *Enteromorpha sp.*, (الطحالب الخضراء)، في محاولة للحصول على بعض التبصر لمستوى التلوث المعدني الذي قد يكون موجودا في البيئة البحرية الساحلية على طول ساحل درنة. تم التقييم من خلال قياس تركيز المعادن الثقيلة كالرصاص، الزنك، المنغنيز و الكادميوم، في أنسجة الطحالب ومياه البحر. تشير النتائج إلى أن تركيزات المعادن كانت أعلى قليلا في *Enteromorpha sp.*, مما كانت عليه في *Cystoseria sp.*, في جميع محطات أخذ العينات. وتركيزات المعادن المسجلة للأنسجة والمواقع المختلفة لهذه الدراسة تؤكد ان التركيزات أعلى عادة في الصيف. وكان متوسط مؤشر التلوث أعلى (0.86) لكل من النوعين في المحطة S6 وأقل (0.29) في المحطة S1. بشكل عام، لا تظهر جميع المعادن الثقيلة أي آثار ضارة على البيئة المائية المحلية لساحل درنة. ومع ذلك، يجب رصدها بشكل مستمر لضمان بقاءها في مستويات غير ضارة.

الكلمات المفتاحية : التلوث، المعادن الثقيلة، المؤشرات الحيوية، مياه الصرف الصحي، الطحالب الكبيرة.

* مسعود زعطوط:marwan2004h@yahoo.co.uk، منظمة زعطوط لحماية الحياة البرية والبحرية في درنة، درنة، ليبيا