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E-mail: Jmac2009m@yahoo.com

P.O,Box (9216) Tel:5238850

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Reactive Oxygen Species Induced by Enterobacteriaceae in Human Uroepithelial Cells

Osama Bassim Al-Saffar

Madenat Al-elem Univ.College,Dept.Biol.,

Al-Kadhmiya ,Baghdad , Iraq

E-mail: O2004B2005@yahoo.com

Abstract

Three hundred mid-stream urine specimens were collected from 300 patients with Urinary Tract Infections (UTI). One hundred and thirty isolates were obtained from mid-stream urines specimens included: *Escherichia coli* (63.84%), *Klebsiella spp.*(23.07%), (*K. oxytoca* 16.15%, *K. planticola* 6.92%). *Enterobacter aerogenes* (6.19%) and *Proteus spp.* (6.92%) (*P. vulgaris* 4.61%, *P. mirabilis* 2.31%). They were identified according to the cultural and biochemical properties. Patients were divided into five groups (A, B, C, D and E) according to the pus cells level in their urine specimens. Moreover, the type and prevalence of bacterial infection in pus cells in each studied group were detected. *E. coli* performed the highest percentage (61.36%) in all studied groups, particularly group A. Also the study includes the assessment of ROS inducing uropathogens which is measured by using malonaldehyde (MDA) method. The results showed that the level of ROS was significantly ($P < 0.05$) increased according to the level of pus cells. Thus, group E showed high level of ROS (9.08 nmol/l) in comparison with other groups in this study. On the other hand, the ability of uropathogens to induce ROS was determined. *E. coli* isolates particularly *E. coli*19 showed a putative efficiency for induction of ROS (11.62 nmol/l). In contrast, *K.planticola*4 exhibited the lowest level of ROS (3.14 nmol/l).

Urinary tract is one of the most common sites of bacterial infection, in general urinary tract infections represent a major health problem in many areas of the world, and it is the most frequently encountered infection in daily practices (1). Many bacteria has ability to cause UTI, such as *E.coli*, *Klebsiella spp.*, *Enterobacter aerogenes*, and *Proteus spp.*, many studies reported that *E.coli* gave the highest percentage in patients beside other species, which found in low percentage (2).

Reactive oxygen species (ROS) are deleterious in excess. They are naturally produced by aerobic metabolism and are a permanent threat to living organisms (3). All organisms have developed ways of protecting themselves against ROS, including specific defenses and global responses that enable cells to survive periods of oxidative stress. Both types of protection are regulated and responded to the environment-associated oxidative threat (4).

ROS instability and inability to permeate lipid membranes usually provide an effective shield against propagating damage. However, through reactions with polyunsaturated fatty acids, they generate lipid hydroperoxides and unsaturated aldehydes, which are highly electrophilic, stable, readily propagating between cellular compartments, and capable of reacting with proteins and nucleic acids (4, 5).

This chain reaction of lipid peroxidation accounts for the role played by ROS in the pathogenesis of atherosclerosis, ischemia, reperfusion injury, and other diseases (5).

The influence of reactive oxygen species (ROS) on cells becomes of increasing interest and the cells damage caused by an excessive production of free radicals or reactive oxygen species (ROS) has been extensively studied. In patients with UTI, an elevated production of ROS in urine and increased ROS-mediated damage of

epithelial cells membranes have been detected (6).

It is now well established that mitochondria is the main site of the generation of oxygen radicals, such as, superoxide anion, hydroxyl radical, singlet oxygen and hydrogen peroxide (7). It is estimated that 1–4% of oxygen reacting with the respiratory chain leads to the formation of superoxide radicals ($O_2^{\cdot-}$). The other sources of reactive oxygen species include radiation, cytotoxic chemicals and antibiotics (4, 7).

Malondialdehyde (MDA) is an indicator of lipid peroxidation which increases in various diseases. This increase is reflected in enhanced excretion of several MDA derivatives in the urine (8).

The aims of this study were:

Determination of induced ROS by uropathogens in uroepithelial cells of patients with UTI.

MATERIALS AND METHODS

Patients and urine samples

A total of 300 of midstream urine specimens from patients with UTI only were collected in 5 ml of sterile container.

Those patients were referred to Al-Yarmouk Hospital during the period from 1st of Jan. 2009 to 1st of April 2009, with symptoms suggesting acute UTI. Inclusion criteria were dysuria, frequency, urgency, and abdominal flank pain with or without fever. Patients receiving antibiotic therapy were excluded from the study.

Culture

A loopful of undiluted urine sample was spreaded on MacConkey and Blood agar. The plates were incubated at 37°C for 18h. After incubation, the growth was observed as well as the ability to ferment lactose. Non-fermentable

colonies were re-incubated on blood agar and incubated at 37°C for 18h (9).

Identification of Bacteria

Small part of selected colony of positive culture was transferred and fixed on a microscopic slide, then stained with gram stain to examine cell's shape, grouping and spore forming then biochemical test we used to complete identification (10).

Assay of MDA

Measuring the malondialdehyde (MDA) by thiobarbituric acid (TBA) reactivity is the most widely used method for assessing lipid peroxidation. Malondialdehyde was estimated according to the modified method by Hunter 1985 (11). The pink color which produced in this method is due to the formation of an adduct between the thiobarbituric acid (TBA) and malondialdehyde under acidic conditions (12).

MDA levels were measured by a spectrophotometer. The reaction mixture contained 0.1ml urine sample, 0.2ml of 8.1% SDS (sodium dodecyl sulfate), 1.5ml of 20% acetic acid, and 1.5ml of 0.8% aqueous solution of thiobarbituric acid. The mixture pH was adjusted to 3.5 and the volume was finally made up to 4.0ml with distilled water and 5.0ml of the mixture of n-butanol was added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the absorbance of the organic layer was measured at 532 nm. MDA level was expressed as nmol/l (11).

Result and Discussion

Isolation and identification

Results of 300 mid-stream urine samples collected from patients suffering from symptoms referred as urinary tract infection showed that 130 (52.0%) specimens have growth on MacConkey and blood agar.

Examination of suspected isolates showed that 63.84% (n=83) of isolates belonged to *E.coli* according to the cultural and microscopical properties. Whereas, 23.07% (n=30) of positive cultures were belonged to the *Klebsiella spp.*

In addition 6.15% (n=8) of isolates were belonged to *Enterobacter spp.* Furthermore, 6.92% (n=9) of isolates belonged to *Proteus spp.*, the colonies appeared a special phenomenon called swarming (table 1).

Biochemical properties of isolates

Table (2) showed the biochemical characteristics of uropathogens isolated from UTI patients.

It was indicated that 63.84% (n=83) of isolates gave clearly positive result for Indole, methyl red, and orthinine, but they were negative for Vogas-proskauer, urease production, Simmon citrate, and oxidase test. On the other hand, these isolates fermented sugar and produced gas on TSI medium but no H₂S was noticed. Thus, these properties were represented *E.coli* (13).

While 23.07% (n=30) of isolates gave clearly positive result on Indole medium, Vogas-proskauer test, produced urea on urea agar, and Simmon citrate medium, but they were negative to methyl red assay (except *Klebsiella planticola* gave positive of this test), orthinine test, and oxidase test. On the other hand, these isolates fermented sugar and produced CO₂ without production H₂S on TSI medium. Therefore, it can be concluded that these isolates represented *Klebsiella oxytoca* and *Klebsiella planticola* according to (13). Notably, *Klebsiella oxytoca* formed 16.15% (n=21) whereas *Klebsiella planticola* formed 6.92% (n=9).

In addition, 6.15% (n=8) of isolates gave clearly positive result for Vogas-proskauer, Simmon citrate, and orthinine assays, and were

negative to oxidase, Indole, methyl red, and urease production test. On the other hand, these isolates able to ferment sugar and produce CO₂ without producing H₂S on TSI medium. These properties related to *Entrobacter aerogenes* (13).

As well as 4.61% (n=6) of isolates were clearly positive to Indole, methyl red, urease production, and Simmon citrate assays, but they were negative for Vogas-proskauer, and oxidase test. These isolates were fermented sugars, produced gas, and H₂S on TSI medium. Thus, these properties were represented *Proteus vulgaris* (13).

In contrast, *Proteus mirabilis* that formed 2.31% (n=3) of isolates was showed negative result to indole test and positive result to orthinine assay.

In general, these isolates considered as uropathogens of urinary tract infection, they have virulent factors such as adherence factors, and endotoxin (13).

Level of pus cells in UTI patients

The specimens were divided into 5 groups according to the level of Pus cells as shown in table (3).

The pus cells give an indication to the severity of urinary tract infections (15).

According to WHO standards, less than 6 pus cells in a urine specimen, it will consider as a healthy. the result indicated that group A has a level of pus cells ranged between (6-10 c.mm) no less no more because if pus cells were less than 6 it considered as healthy group (16).

Moreover, this group was formed (33.80%) of 130 specimen, and number of patient in group A was appeared with significance differences in comparison with all groups at (P<0.05).

In regard to group B, the result showed increasing in the total number of patients, 48 (36.90%). With significance differences in number of patients when compared with groups A, C, D, and E respectively at (P<0.05).

In the group C the number of patient was significantly decreased ,(13.10%) when compared with the above groups, and appeared with significant differences in number of patients when compared with groups A, B, D, and E, respectively at (P<0.05).

Group D showed significant decreasing in the incidence (7.7 %),and gave significant differences in number of patients in comparison with groups A, B, and C, respectively, but not significant with E group at (P<0.05).

While group E was considered the highest group that exhibited high level of pus cells (over 40 c.mm) with low percentage in incidence among UTI cases 8.50% (n=11).Group E was showed significant differences in number of patients in comparison with groups A, B, and C, respectively, but not with group E (P<0.05).

MDA associated with level of pus cells

Table (4) showed the level of MDA, in each studied group of UTI, associated with level of pus cells. Group A revealed low level of MDA, it was 4.75 nmol/l. Thereafter, the level of MDA was increased significantly due to increasing pus cells level, thus group E represent the highest value of MDA concentration (9.08nmol/l) in comparison with other groups.

Although the level of MDA in group A was low, it achieved a significant difference with other groups and control at p<0.05.

In Group B, the level of MDA was significantly (p<0.05) increased (5.76 nmol/l) when compared with other groups and control (2.76 nmol/l).

Obviously, the level of MDA was increased in group C, D and E (7.21, 8.03, and 9.08 nmol/l, respectively). This increasing was significant in comparison with other groups and control at $p < 0.05$.

The efficient of bacterial isolates for the induction it MDA of patients with UTI

Figure 1 showed the ability of *E.coli* isolates to induce ROS in UTI patients. Markedly, the isolate *E.coli*19 had high efficacy for induction of ROS in comparison with other isolates; the concentration of MDA was 11.62 nmol/l. In contrast, *E. coli*3 exhibited less efficiency for induction MDA, the concentration of MDA was 3.51 nmol/l.

Figure (2) showed the ability of *E.aerogenes* isolates to induce MDA in UTI patients. Obviously the isolate *E.aerogenes*4 the highest efficiency for the induction MDA in comparison with other isolates; the MDA was 7.55 (nmol/l). In contrast, *E.aerogenes*1 induced low level of MDA, ration of MDA.

Figure 3 showed the ability of *K.oxytoca* induce ROS in UTI patients. The isolates *K. oxytoca*19 and *K. oxytoca*11 expressed high level of MDA (8.21, and 8.12 nmol/l, respectively). On the contrary the isolate *K. oxytoca*2 induced low level of ROS; (4.35 nmol/l).

In addition Figure 4 shows ability of *K.planticola* to induce ROS in UTI patients. The isolates *K.planticola*8 showed the highest level of MDA (6.86 nmol/l), while the isolate *K.planticola*4 expressed the lowest level of MDA (3.14 nmol/l).

Figure (5) shows the ability of *P. mirabilis* to induce ROS in UTI patients. The isolate *P. mirabilis*3 revealed high level of MDA (7.32 nmol/l), while the *P. mirabilis*2 isolate shows low level of MDA was (5.51 nmol/l).

Figure 6 showed the efficiency of *P.vulgaris* for the induction of ROS in epithelial cells in patients with UTIs. It was indicated that the isolate *P. vulgaris*5 exposed the highest level of MDA; the concentration of lipid peroxidation was 7.97 nmol/l. The next was isolate *P. vulgaris*4 which showed higher level of MDA than *P. vulgaris*2; the concentration of lipid peroxidation was 7.62 and 6.19 nmol/l, respectively. Whereas the isolates *P. vulgaris*6, *P. vulgaris*3 and *P. vulgaris*1 revealed low level of MDA, it was 5.63, 5.42 and 4.03 nmol/l respectively.

In general, the results showed the reactive oxygen species (ROS) in UTI patients was higher than healthy person haven't any bacterial infection, also in this study showed the healthy groups haven't high level of ROS, for this reasons agreed with previous study (17,18).

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Table (1) the cultural and morphological characteristics of uropathogen isolated from patients with UTI

Bacterial Isolate	No. of Isolates	Percentage of total isolates %	Cultural Properties on		Morphological Characteristics	Motility
			MacConkey agar	Blood agar		
E.coli	83	63.84	Pink colony	White colony	Cocccobacilli or Bacilli	+
Klebsiella spp.	30	23.07	Pink colony mucoid	White colony	Bacilli	-
Enterobacter Spp.	8	6.15	Pink colony	White colony	Bacilli	+
Proteus spp.	9	6.92	Pale colony	Swarming	Cocccobacilli	+

+Positive (motile)

-Negative(Non-motile)

Table (2) Biochemical properties of Uropathogens isolates from UTIs patients

Bacterial Isolates Tests	<i>E.coli</i>	<i>Klebsiella oxytoca</i>	<i>Klebsiella planticola</i>	<i>Enterobacter aeorgenes</i>	<i>Proteus mirabilis</i>	<i>Proteus vulgaris</i>
Oxidase	-	-	-	-	-	-
Indole	+	+	-	-	-	+
Methyl Red	+	-	+	-	+	+
Vogas Proskawer	-	+	+	+	-	-
Simmon Citrate	-	+	+	+	+	+
Urease	-	+	+	-	+	+
TSI	A/A+-	A/A+-	A/A+-	A/A+-	A/A++	A/A++
Ornithine	+	-	-	+	+	-

(+) Positiveresult

(-) Negative result

A\A ++ (yellow slant (acid), yellow buttacid, and no gas or H₂S production).

A\A + - (yellow slant (acid), yellowacidbutt, gas production,and no H₂S formation).

Table (3) Grouping of pus cells in urine sample from patients with UTI

Level of Pus cell in specimens			
Groups	Pus Level c.mm	Number of patients	Percentage %
A	(6-10)	44 a	33.80%
B	(11-20)	48 b	36.90%
C	(21-30)	17 c	13.10%
D	(31-40)	10 d	7.70%
E	(Over 40)	11 d	8.50%
Total		130	100%

- The identical small letters refer to non- significant differences between number of patients in each row at $p < 0.05$ level.

Table (4) the level of MDA in UTI groups

Groups	Mean MDA of concentration (nmol/l) \pm SD
A	4.75 \pm 0.60 a
B	5.76 \pm 0.59 b
C	7.21 \pm 0.54 c
D	8.03 \pm 0.53 d
E	9.08 \pm 0.30 e
Controls	2.76 \pm 0.17 f

- The identical small letters refer to non- significant differences between mean of MDA and controls in each row at $p < 0.05$ level.

\pm SD: Standard Deviation

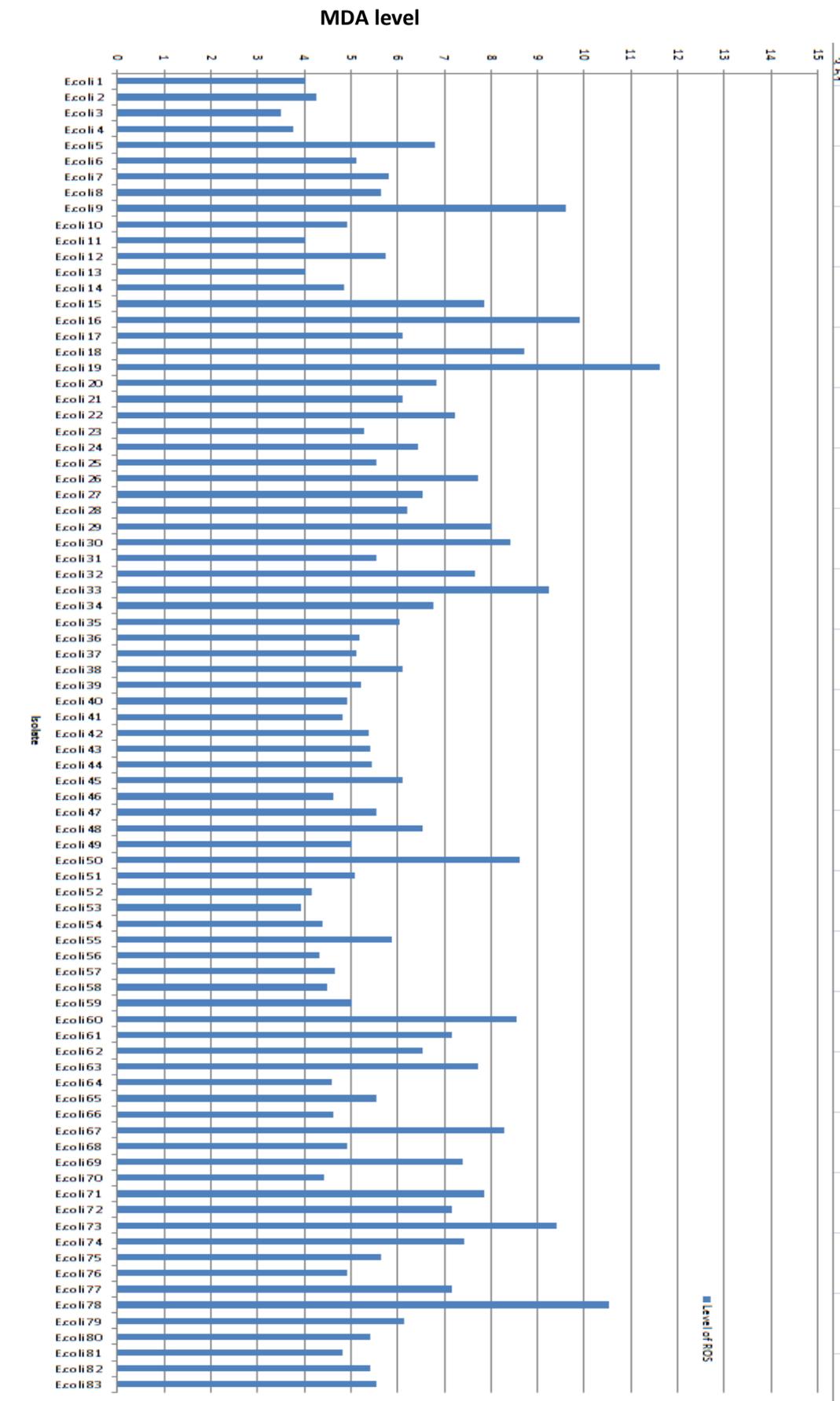


Figure (1) MDA level induced by *E. coli* isolates in UTI patients

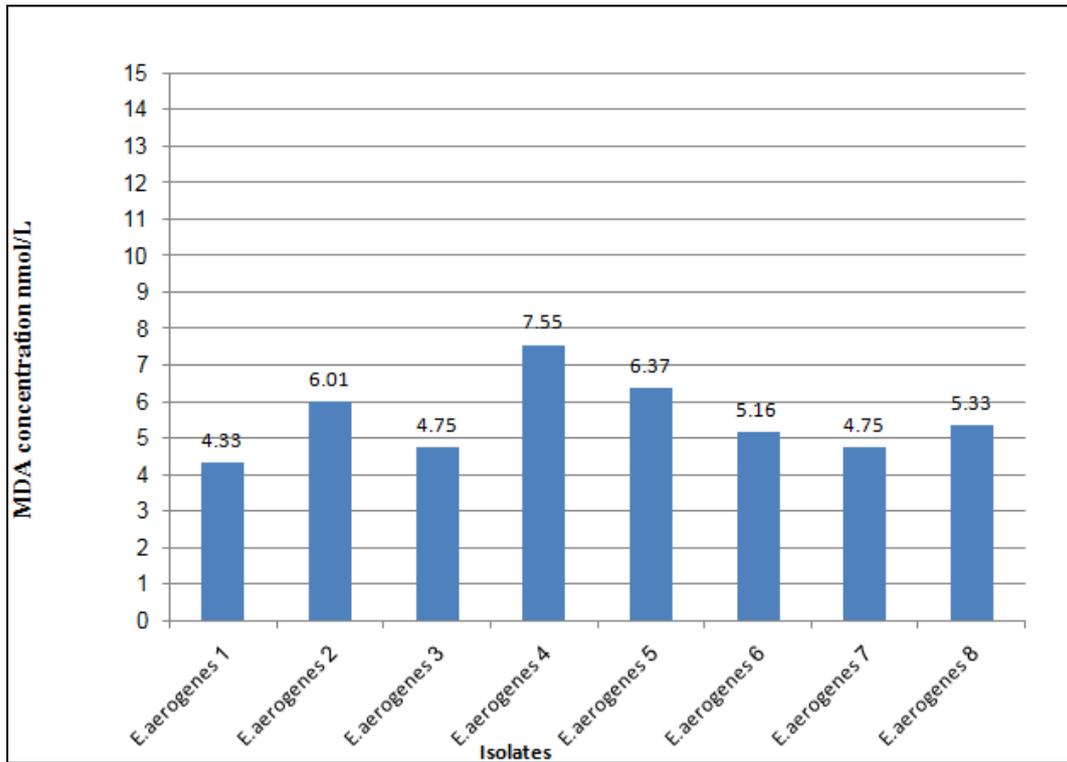


Figure (2) MDA level induced by *Entrobacter aerogenes* isolates in UTI patients

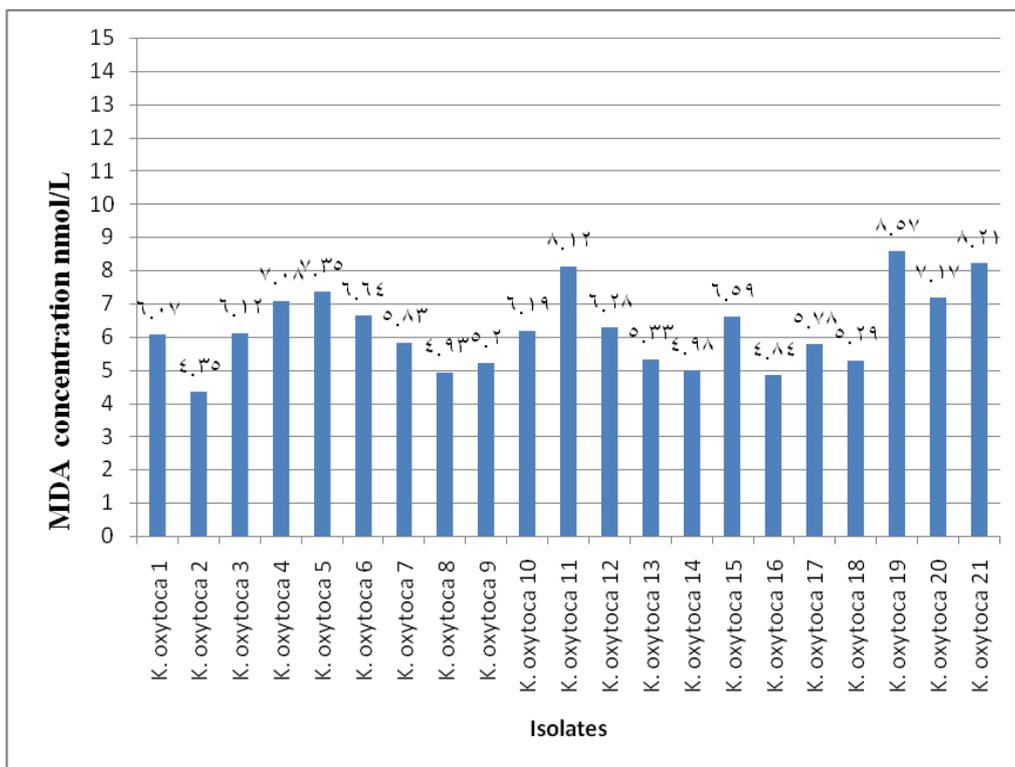


Figure (3) MDA level induced by *Klebsiella oxytoca* isolates in UTI patients

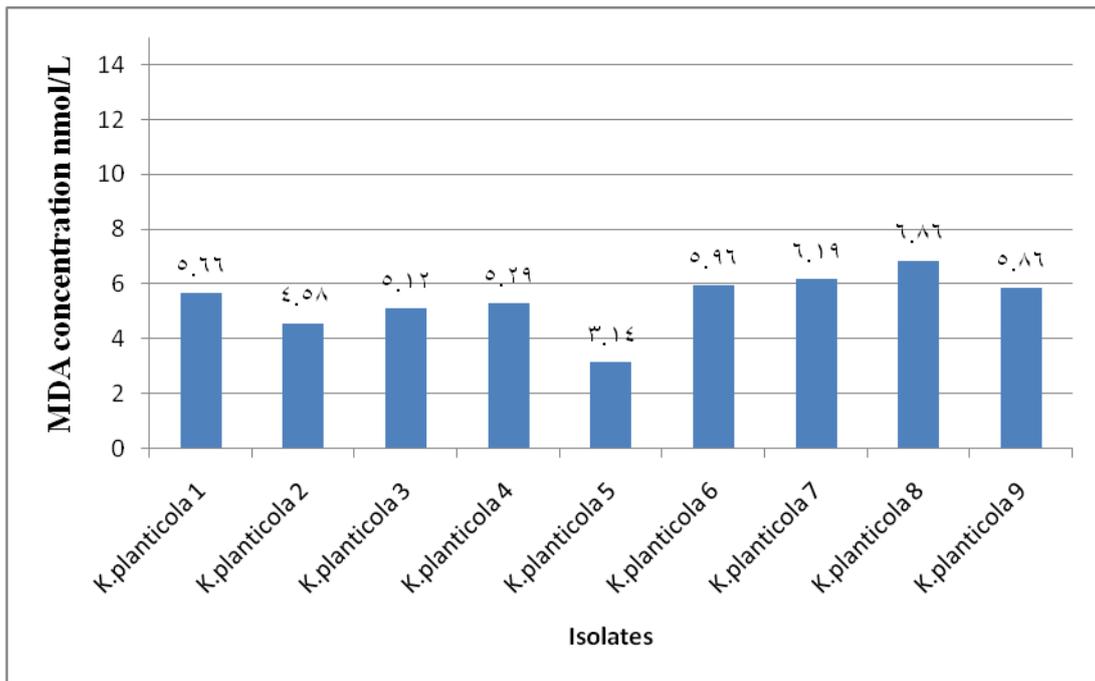


Figure (4) MDA level induced by *Klebsiella planticola* isolates in UTI patients

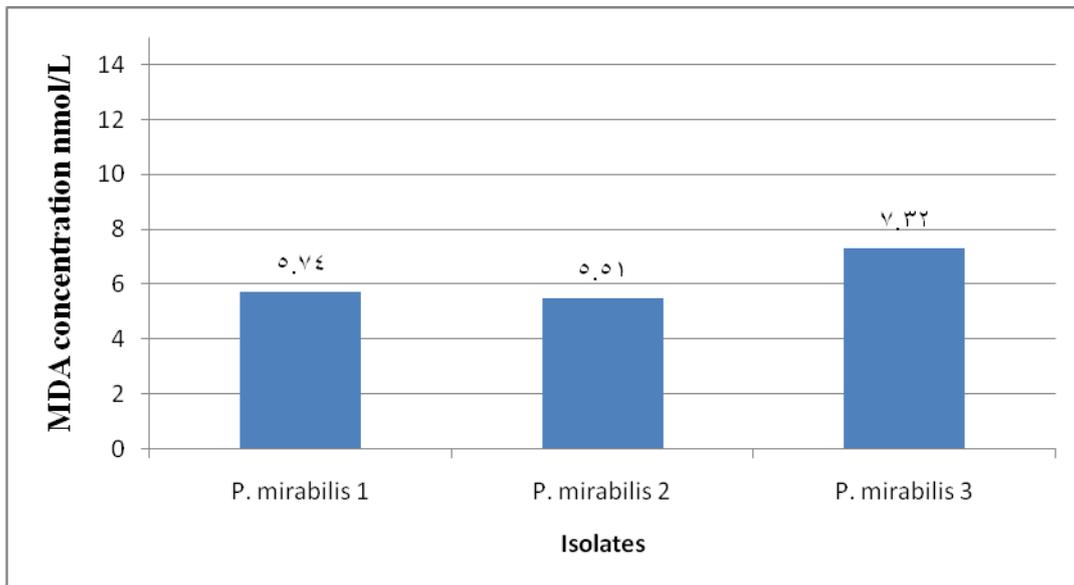


Figure (5) MDA level induced by *Proteus mirabilis* isolates in UTI patients

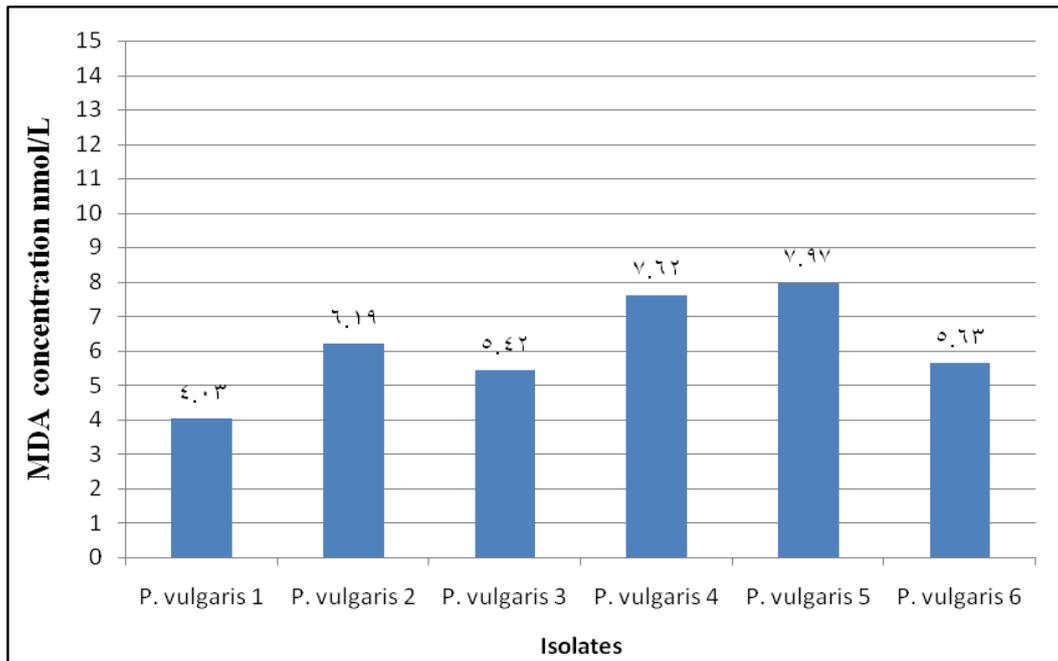


Figure (6) MDA level induced by *Proteus vulgaris* isolates in UTI patients

الخلاصة

جمعت ثلاثمئة عينة ادرار من مرضى يشكون من اخماج المجاري البولية. تم الحصول على مئة وثلاثون عزلة من عينات الادرار وتضمنت : *Escherichia.coli* بنسبة ٦٣,٨٤% و بكتريا *Klebsiella spp.* ٢٣,٠٧% (*K.Planticola* ٦,٩٢% و *K.oxytoca* ١٦,١٥%) وبكتريا *Entrobacter aerogenes* بنسبة ٦,١٥% واخيراً بكتريا *Proteus spp* بنسبة ٦,٩٢% (٤,٦١% *P.mirabilis* و ٢,٣١% *P.vulgaris*). شخّصت العزلات بالاعتماد على الصفات الزرعية المظهرية البايوكيميائية. تم تقسيم المرضى الى خمس مجموعات (A,B,C,D,E) اعتماداً على اعداد الخلايا الالتهابية الموجوده في عينات الادرار. تم التحري عن نوع ونسب تواجد البكتريا الممرضة في الخلايا الالتهابية، لوحظ ان بكتريا *E. coli* قد شكلت النسبة المئوية العالية في كل المجاميع المدروسة وخصوصاً المجموعة A (التي اعطت ٦١,٣٦%)؛ فضلاً عن ذلك، شملت الدراسة على تقييم قابلية الممرضات البولية في حث ROS والذي قيس بالاعتماد على طريقة *Malonodialdehyde (MDA)*. وقد اكدت النتائج ان مستوى *MDA* قد ازداد معنوياً (بمستوى $P<0.05$) طبقاً لزيادة الخلايا الالتهابية، لذا لوحظ ان المجموعة E قد اظهرت زيادة في *MDA* (٩,٠٨ نانومول / مل) مقارنةً بالمجاميع المدروسة من جانب اخر، تم تحديد قابلية الممرضات البولية في حث ROS. وكانت عزلات *E. coli* الاكفأ في حث ROS وخصوصاً العزلة *E. coli 19* التي اظهرت تركيزاً عالياً من *MDA* وبلغ ١١,٦٢ (نانومول/مل)، على العكس فقد سجلت العزلة *K.planticola* مستوى منخفض من *MDA* بلغ ٣,١٤ (نانومول/مل).

Hazard of heavy metal resistant bacteria in polluted water and soil

* Eman Hindy Katie ,** Jabbar Farhan Al-maadhidi, Nibal Khalil Mousa

* Ameena Ghazi Abid.

*Ministry Of Science and Technology.

**Madenat Alelem University College.

Abstract

Fifty two Chromate resistant bacterial strains were isolated from industrial sewage contaminated with Chromate, different soil sample, polluted river sediment, and many hospitals sewage. The isolates showed resistance to concentrations of Chromate, which was available as $K_2Cr_2O_7$ or H_2CrO_3 .

Forty two isolates were gram negative *bacilli* and the other gram positive *bacilli*.

In addition to resistance of Chromate as a heavy metal, they were able to resist some antibiotics. The results showed that most isolates were antibiotic multi-resistant.

Growth curve of selected isolates showed an obvious decline at high concentrations of Chromate.

Introduction :

During last few decades extensive attention has been paid to the hazard arising from contamination of the environment by heavy metals. Heavy metals are major pollutants in marine, ground, industrial and even treated water(1).

Microorganisms can play an important role in the detoxification and / or removal of heavy metals from polluted environments.

A wide variety of fungi, algae and bacteria are now under study or already in use as biosorbents for heavy metal remediation.(2) Heavy metals are such as Chromium, mercury and copper are found among hydrocarbons and increases the difficulty of biodegradation.

Lower cost and higher efficiency at low metal concentrations make biotechnological processes very attractive in comparison to physicochemical methods for heavy metals removal.

Chromium is one of the most toxic and carcinogenic heavy metals. Divalent and trivalent chromium species are the most stable and least toxic ones , while hexavalent chromate is highly toxic, readily crossing the membrane of eukaryotic and prokaryotic cells, causing oxidative cellular damage .(4)

Chromium compounds are widely used, e.g., in leather tanning, metal finishing, alloy preparation and wood preservation. Wastes are often, discharged to the environment, especially in countries which impose inadequate regulatory control; the need for effective, economic waste remediation at source is urgent.

The aim of the present work are to study some bacterial strains isolated from oil and metal polluted sites regarding the capability concentration for some heavy metals and also to identify the microbial

community of resistant bacteria of metal – contaminated sites.

Chromate is soluble and thus readily spreads beyond the site of initial contaminants convert chrome to Cr(III), which is much less toxic and less soluble ;therefore, bioremediation of chromate is of considerable interest, especially given the chemical means are prohibitively expensive for large scale cleanup.(6)

Materials And Methods:

Collection of samples:

Water and soil sampling were collected from contaminated sites, industrial slug, sediment of Deala river, hospital sewage, samples from the surface and different depth of soils, were taken as sources of heavy metals resistant bacteria. These samples were stored in sterile glass bottles at 4°C for further work.

Preparation of samples:

Samples were mixed with 10 volumes of distilled water, then serially six fold diluted, and plating diluted samples on N. Agar supplemented with 0.1mM of chromate colonies were counted after 3 days incubation at 30°C . Cell growth was measured as g/L after centrifugation ,precipitating and drying. These strains were purified on N. agar without chromate.

The bacterial strains were then tested for their ability to grow on Macconkey agar, EMB, cetrimide agar with or without chromate (0.1mM,) which supplemented as K₂Cr₂O₇ or H₂CrO₃ ,percentage of living bacteria measured by counting living cell in culturing media with or without chromate.

Resistant isolates were cultured on L. agar for screening of antibiotics resistance by disc method culture.

Antibiotics Test:

- *Gramicidin.
- *Ramfabin.
- *Amoxicillin.
- *Erythromycin.
- *Carbencin.
- *Nalixin.
- *Tetracycline.
- *Chloramphenicol.

Antibiotic resistance was estimated by observing the clear zone around growth of colonies after (24-48 hr) of incubation at 30C°. Selected isolates were cultured on L. agar supplemented with different concentrations of chromate to estimate bacterial growth.

Result and Discussion:

Different samples of contaminated locations with chromate like, sewage sludge . Industrial sewage municipal disposal were used as source of resistant bacteria to the heavy metal. The chromate sources were H₂CrO₃ and K₂Cr₂O₇ .

The experiments were carried out in duplicate and each source was also assayed in duplicate .

The number of colony forming unit (CFU) in the samples was determined by making samples with 100 ml of D. water and plating

on 0.1Mm Cr supplemented medium (Nutrient Agar, EM. Cetrinide, MacConkey).

Colonies were counted after 2 days incubation at 30C°. The same media without Chromate used as a control .

All samples from polluted area showed different strains at many percentage value of bacteria to the primary concentration of chromate(0.1Mn)resistant (1,2).

It can be observed that the strains isolated from the metal contaminated sites were the most tolerant to Chromate. using microorganisms as biosorbents for heavy metals offers a potential alternative to existing methods for detoxication and for recovery of toxic or valuable metals from industrial discharge water.

Many bacteria including *Pseudomonas sp.* *Enterobacter*, showed high resistant to Chromate, especially those were originally isolated from industrial sewage polluted with Chromate (1,2) .

Pseudomonas sp., and *rhodococcus* strains presented same resistant to heavy metals used, being suitable for use in sites contaminated with high concentrations of them .(7)

Municipal sludge often contain high quantities of toxic metal and other compounds that must be removed.(8 , 9)

The sewage sample of hospitals contains bacterial strains have multi –resistant of antibiotic in addition to its resistance to heavy metals. tab (3)

Municipal sludge contain high quantities of contaminants, such as toxic metal,

pathogenic organisms and hazardous organic compounds.(6 , 7)

Eleven bacteria strains were selected depending on its growth activity on chromate containing media tab(3).These isolated for survival with different antibiotics tab(4)using antibiotics disk method selected isolates, (MC2 , Cr15 ,SW, SW13, SW4)were tested for their ability to grow in many concentration of chromate by culturing them in specific media containing chromate concentration of (1-10)Mm.

All strains presented an efficient capability to resist Chromate at concentration (1,5) mM but they were sensitive to it at 10mM.

Bacterial strains identified as gram negative *bacillus* and one as a strain of gram positive *bacillus* .All of these strains isolated from industrial hospital sewage respectively were resistant up to (5mM) Chromate, but they were not tolerance to Chromate at 10mM conc. Many bacteria, including *Pseudomonas sp* , *E. coli*, and *Enterobacter* can reduce (Cr VI) to the less toxic Cr (III), which readily precipitates as Cr(OH)₃.(10 , 11)

Therefore it is possible; use these tolerant microorganisms to the Chromate at heavy metal site that is being bioremediated. It can observed that the strains isolated from the metal contaminated sites were the most tolerant to Chromate.

Medium	Industrial sewage (sludge)	Soil	Hospital Sewage
Nutrient agar	85	78	65
MacC.(f)	100	100	68
MacC n.f.	100	100	87
E.M.B.	80	80	60
Cetrimide	100	100	50

Tab.(1) Percentage of chromate resistant bacteria

K₂Cr₂O₇ 0.1mM

Medium	Industrial Sewage (sludge)	Soil	Hospital Sewage
Nutrient agar	95	80	48
MacC.(f).	75	72	68
MacC . nf.	95	92	46
E.M.B.	70	86	56
Cetrimid	80	78	70

Tab. (2) Percentage of chromate resistant bacteria

$H_2.CrO_3$ 0.1mM

isolate	Growth g /L	
	H_2CrO_3	K_2CrO_7
Mc ₂	2.4	2.5
Mc ₃	1.8	0.9
Mc ₈	1.3	1.0
Cr ₅	1.4	1.0
Cr ₇	1.4	1.8
Cr ₁₅	2.9	2.5
Eb ₆	1.5	1.2
Eb ₁₁	1.2	0.8
Sw ₆	1.2	0.8
Sw ₁₃	2.5	2.6
Sw ₄	2.6	2.6

Tab(3) Chromium resistant bacteria growing on chromate supplementing media with concentration of 0.1mM

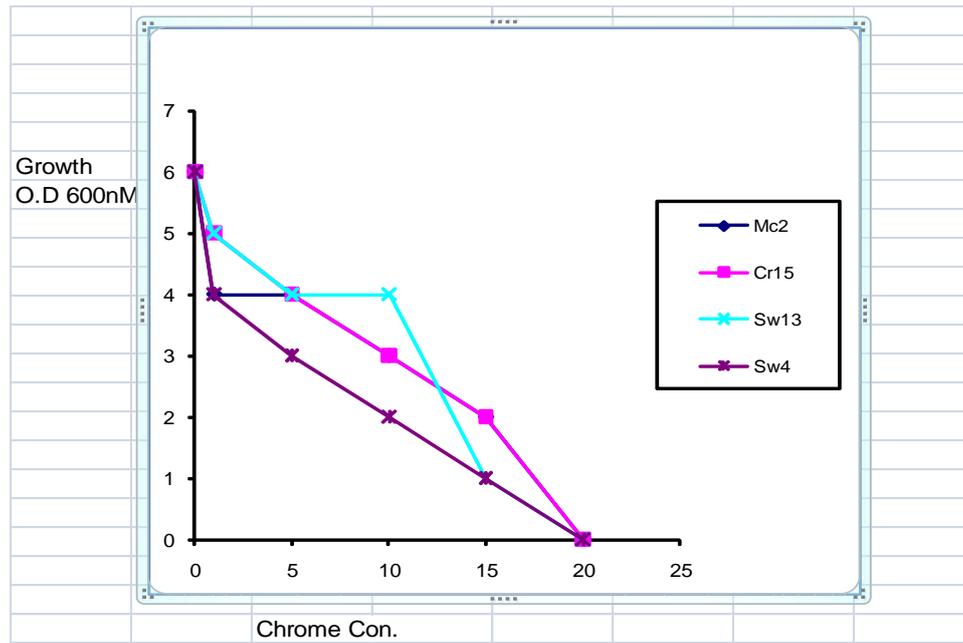


Fig.(1)Growth curve of chromate resistant isolates.

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مخاطر البكتريا المقاومة للمعادن الثقيلة في تلوث الماء والترربة

*ايمان هندي كاطع ،**جبار فرحان المعاضيدي ،*نبال خليل موسى*،امينة غازي عبود

*وزارة العلوم والتكنولوجيا .

**كلية مدينة العلم الجامعة.

الخلاصة

تم الحصول على 52 عزلة بكتيرية مقاومة للكروم عزلت من المخلفات الصناعية ، نماذج تربة مختلفة ، رواسب نهر ملوثة (جسر ديالى) ومياه مجاري من المستشفيات . أظهرت العزلات مقاومة للتراكيز المختلفة للكروم الذي تم تجهيزه للعزلات بشكل مادتي الداكرومات $K_2Cr_2O_7$ وحمض الكرومك H_2CrO_3 .

بينت الفحوصات النظرية والميكروسكوبية ان 42 عزلة هي عصيات سالبة لصبغة كرام والاخرى هي موجبة لصبغة كرام، العزلات المختارة أظهرت مقاومة للتراكيز الأولية للكروم من خلال منحنى النمو وانخفاض واضح في التراكيز العالية التي تصل إلى 10 mM .

Short Communication**Digestion of Fiber and
Increased Crude Protein in Corn Cob**

J. F. Al-maadhidi

*M. T. Al-khatib

Biol. Dept.,

Dept. of Indust. Microbio, Food &

Madenat Al-elem Univ. College.

Biotech. Center, MOST.

Corn cob is agricultural by products rich in cellulosic fibers and poor in protein contents (1), therefore to make it available for feeding of ruminants, fishes and poultry need processing to decrease the fibers and increasing the protein contents. Chemical digestion and aerate fermentation had been conducted. Crushed, milled corn cob (0.1 – 0.5 cm) was soaked in 1% NaOH solution and autoclaved for 30 minutes at 121C° and 1.5 Lb/cm³. After cooling, fermentation experiment of 10 % suspended corn cob in distilled water was adjusted to pH 5 and 4% molasses, 1g/L urea were added, inoculated with 50ml/L yeast culture as initial were conducted in Kell fermentor of 20L working capacity under controlled conditions (30 C°, 150 rpm, pH 5 and aeration 2m³/h), experiments duration were 72hrs. Fermentor contents were dried in electric oven at 70 C°. Dried material was homogenized and analyzed for protein content using microkeldal method and for fiber determination using Infra analyzer instruments. Results from digestion and fermentation experiments revealed significant decrease in fiber contents, it was decreased from 41.7% in untreated control to 7.58 ± 1.7%, while the protein was increased from 2.4% in nonfermented control to 16.3%±2.57 in product of fermentation experiment (table - 1). These results indicate a highly improved in feeding quality of corn cob, which can be used successfully for ruminant, fishes and poultry feeding.

Table (1): Percentages of protein and fibers in processed and fermented corn cob

Batch No.	protein%	- x	fibers%	- x
Untreated control	2.4	2.4	41.7	41.7
R20	19.7		6.4	
R21	18.3	16.3±2.57	6.3	7.58±1.72
R22	13.9		9.2	
R23	15.2		6.3	
R24	14.3		9.7	

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الخلاصة

إنّ معالجة مسحوق كوالح الذرة الصفراء كيميائياً وبيولوجياً أدى الى انخفاض معنوي في محتواها من الألياف وزيادة معنوية في كمية البروتين الخام، مما يعني إمكانية استخدامها بنجاح في تغذية الحيوانات المجترة والأسماك والدواجن دون أية أعراض جانبية.