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Articles need to contain the following items:

- Abstract in English and Arabic not more than 300 words.
- Article includes the following items: Introduction, Materials and Methods, Results and Discussion, Conclusion and References.
- References should be numbered in the text according to the sequence appeared in the text and listed in order.
- Tables and figures should be appropriately titled with size not exceed an A4 page.
- The editor reserve the right to refuse or accept any article submitted.

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Histological Structure of the integument in *Mystus pelusius (Solander)*

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Abstract

The histological structure of the integument in *Mystus pelusius*_have been investigated. The study based on the micro- anatomical investigation of the skin fragments taken from nine specific regions of the body.

The results of the present study revealed that the integument comprise three principal layers, represented by the epidermis, the dermis (corium) and the subcutis. The epidermis may further be divided into the outer most epithelial layer (coverage), the middle layer and the basal layer (stratum germinativum) .The thickness of the epidermis in the investigated fish varies to a great extend (50-250 µm).

The dermis of *M. pelusius* is consisting of a relatively thin upper layer of loose vascular connective tissue called the stratum laxam and a thic lower compact layer called stratum compactum. The thickness of the dermis showed a great exterd (25-250 µm).

The subcutis is the inner most and almost the thinnest layer of the skin. It shows some empty spaces which are occupied by fat cells. The thickness of the subcutis layer in *M. pelusius* is ranged from 38 µm to 175 µm.

التركيب النسيجي للجلد في سمكة ابو الزمير

Mystus pelusius (Solander)

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قسم علوم الحياة ، كلية التربية (ابن الهيثم)

الاعظمية ، بغداد – العراق

***قسم علوم الحياة ، كلية العلوم للبنات**

الجادرية ، بغداد – العراق

الملخص

تم دراسة التركيب النسيجي للجلد في سمكة ابو الزمير *Mystus pelusius* .
استندت الدراسة الى بحث التشريح الدقيق لقطع من الجلد في تسع مناطق خاصة بالجسم . اوضحت النتائج ان الجلد يتكون من ثلاث طبقات اساسية ممثلة بالبشرة والادمة والطبقة تحت الجلدية . والبشرة قد تقسم الى طبقة خارجية ظهارية مغطية وطبقة وسطى وثالثة قاعدية (طبقة مولدة) إظهر سمك البشرة في السمكة المدروسة تبايناً كبيراً في مدى السمك (٥٠-٢٥٠ مايكروميتر) . الادمة هي الاخرى تتكون من طبقة علوية نحيفة نسبياً من نسيج ضام مفكك وعائي تدعى بالطبقة المنحلة (المفككة) ، واخرى سفلية سميكة مكتنزة تدعى بالطبقة المتراصة ، وسمك الادمة اظهر ايضاً مدى واسع في سمكة (٢٥-٢٥٠ مايكرومتر) . الطبقة تحت الجلدية وهي الداخلية تكون انحف الطبقات وهي تظهر فسح مشغولة بخلايا دهنية ، وهي اسوة بالطبقات الاخرى تظهر تبايناً في السمك (٣٨-١٧٥ مايكروميتر) .

Introduction

The integument is one of the largest organs of the body, making up some 15% of the human body weight(1). The different layers of the integument form some of the most varied structures found within vertebrates , as they are producing several structures which are representing the integument derivatives(1,2,3) .

A review of the literature shows that quite extensive work has been done on the integument of different species of fishes(4,7,8,9,10,11) The present paper report part of an extensive study which has been carried out in an attempt to study the histological structure of the integument of different Iraqi freshwater fishes. One of the major drawbacks with the current investigation the almost lack of scientific data about the histological structure of the integument of the Iraqi freshwater fishes with the exception of the preliminary data reported by Jasim and AL-Daham(12) and Hamed(13).

Materials and Methods

The specimens of *M. pelusius* for this study were collected from the central marshes near AL-chebaesh city at Thiqar province.

Integument fragments of about 1×1 cm were cut from nine regions of the body, (regions listed in table 1) and fixed in 10% formalin. Ethyl alcohol was used as a dehydrating agent. Paraffin sections were cut at 6 µm and were stained by Harris haematoxylin and counter stained with eosin (H & E) .Periodic Acid Schiff (PAS) was also used to recognized the basement membrane. Masson Trichram stain (MTC) was also used to differentiat the different structures and layers of the integument of the fish under investigation. All the above materials prepared and used according to Humason,(14) and Bancroft and Stevens(15).

Results

The integument of *M. pelusius* comprises three main layers- the epidermis, the dermis (corium) and the subcutis. The epidermis and the dermis are separated by a basement membrane which follows the depressions and elevations of the upper surface of the dermis (Figure 1).

The epidermis of *M. pelusius* may further be divided into three layers. These were the outermost epithelial layer, the middle layer and the basal layer (germinativum layer). The thickness of the epidermis of *M. pelusius* varies to a great extent (Table 1).

The stratum germinatirum layer is composed of a single layer of cuboidal cell. Each such cell is provided with oval or spherical nucleus placed in the center of the cell. The cuboidal cells layer situated just above the basement membrane (Figure 1).

In between the cuboidal cells found small oval or round lymphatic spaces which contain lymphocytes with deeply stained nuclei. The middle layer of the epidermis is composed elongated epithelial cells with oval or spherical nuclei. In between these elongated cell found many mucous and club cells which are differ in their number in different integument regions of the body and they have differ measurement in their length and width. The club cells appeared with single centrally placed nucleus or two, sometime three and rarely four and the cytoplasm appear homogenous in rotine stain (H&E) (Figures 2,3,4).

The outermost layer is composed of rather flattened cells arranged in two to six layers (upper and lower lips, 3-6 layers ; head, 2-4 layers; dorsal surface, 2-4 layers; ventral surface, 3-5 layers; at lateral line within the trunk region 3-5, layers; caudal peduncle 5-6, layers and caudal and pectoral fins, 2-3 layers). Goblet cells which are opened to the surface of the body represented clearly in this layer of epidermis (Figure 5).

The dermis consists of relatively thick outer loose connective tissue layer (stratum laxum). The loose connective tissue layer situated below the basement membrane is richly supplied with blood capillaries (Figure 4).

The second or deep layer of epidermis is relatively thin layer and is characterized by the presence of coarse compactly arranged bundles of collagen fibers. The thickness of dermis in *M. pelusius* varies to great extend (25-250 µm).

Histological examination showed that there are number of dermal papillae, which are penetrate deep into the epidermis. These papillae are usually straight and cylindrical in shape and do not break through the basement membrane and the germinal layer

of the epidermis. Dermal papillae appear above the dermal stalk and they are richly supplied with nerves and blood capillaries and lie freely in the epidermis (Figures 2,4). Pigment cells are also found in these papillae.

Taste buds are pear shaped structures found either singly or in groups of two or three situated in certain specialized area of epidermis above the supported dermal papilla. The taste bud formed basal cells, sensory hair cells & supporting cells (Figure 2 &4).

The subcutis layer is bind the stratum compactum with undelying muscle bundles.It is richly supplied by nerves and blood vessels (Figure 6). The sub cutis layer is also varies in its thickness (38-175 µm)

Sections of the integument at lateral line system within the trunk region showed that the trunk canal of lateral line situated in stratum laxum of the dermis. It's opening surrounded by regular dense connective tissue and lined by stratified squamous epithelium. The trunk canal surrounded by white fibrous cartilage (Figures 6 and 7). The neuromast situated deeply in the canal and appear oval in its shape. It is formed from two parts, the first consist of two rows of cells (Sensory hair cells and supporting cells). The second part of the neuromast represented by cupula (Figures 8 and 9).

Discussion

The integument of *M. pelusius* comprise three principal layers which are the epidermis, the dermis and the subcutis. The subcutis is present in all fishes except in *Amia calva* (16,17,18,19). Liem(20) and Mittal and Munshi (4,21), have recognized the subcutis as one of principal layers of the integument in different fish species.

Mittal and Munshi(4) stated that the presence of lymphatic spaces in the stratum germinatirum of the epidermis in teleostean fishes is interested, as they supply nutrition to the stratum germinativum for cell proliferation and protect the epidermis from microorganism or foreign protein. The appearance of a large number of lymphocytes indicate the pathological condition(4). Results of the present study showed few lymphatic spaces with lymphocytes within the stratum germinativum layer. It is not unexpected as the

fish collected from relatively clean water.

Review of the literature declare that the epidermis of fishes is usually equipped with various types of unicellular glands which secret a slippery, gluey viscous substance that keeps the body of the fish slimy. These literature described the different types of slime cells in the epidermis of various fishes, these were : (a) beaker-shaped or flask-shaped cells (Becherzellen), (b) club cells (Kolbenzellen or Kolbenformige gebilde), and (c) sacciform cells (Sack formige seröse drüsen) (4,22,23) . The slime cells found in the epidermis of *M. pelusius* are of various shapes and sizes. They were represented by small goblet cells situated in the outermost layer of the epidermis; mucous cells which are larger in their sizes and concentrated in the middle layer of the epidermis and the third type is club cells which are appeared clearly in the middle layer of the epidermis.

In the dermis if the fish under investigation two distinct layers, the stratum laxum and the straturm compactum may be distinguished. The first is formed from loose connective tissue situated below the basement membrane and it is richly supplied with blood capillaries. The second is formed from compactly arranged bundles of collagen fibers. In the present study the results dealing with the histological structures of the dermis agree with the results reported by several researcher in different species of fishes (4,13,17,18,21,24,25, inter alia).

Examination of the dermis layers in investigated fish showed that there are numbers of dermal papillae which are straight and cylindrical in their shaped and are penetrate deep into the epidermis. They are richly supplied with nerves & blood capillaries and lie freely in the epidermis. This result agree with the data reported by several workers such as Mittal & Munshi(4), who are worked on *Heteropneustes fossilis* , *Amphipnous cuchia* and *Mastacembelus punctatus* ; Hamed (13) and AL- Nakeeb (30), who were working on different Iraqi fish species.

Results of the presents study showed that there are numbers of taste buds in the epidermis of *M. pelusius* in different regions of the body. Each taste bud formed from sensory hair cells, supporting cells & basal cells. This result supported

by the foundation of Lance(26); Hamed(13) and AL- Nakeeb(30).

Results of the presents study showed that the trunk canal of lateral line system situated in stratum laxum of dermis and it is surrounded by white fibrous cartilage(5,30).

The neuromast situated deeply in the canal and appear oval in its shape. The canal neuromasts consist of two main portions (a) the first represented as a cellular portion which composed of two types of cells (hair sensory cells and supporting cells) and (b) the second represented as cupula which embedded in canal cavity. Such results agree with the results obtained by several researchers(27,28,29,30).

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Table (1): The thickness of the epidermis, dermis and subcutis in *M. pelusius* at different region of the body.

Region	Range and mean (-)/µm		
	Epidermis	Dermis	Subcutis
Upper Lip	125 (172) 213	75 (117.5) 175	Undifferentiated
Lower Lip	100 (168.8) 250	50 (106) 150	Undifferentiated
Head	100 (134.4) 175	150 (193.7) 250	50 (61) 75
Dorsal surface	100 (134.3) 163	88 (102.5) 130	50 (77) 100
Ventral surface	125 (153.7) 175	125 (192) 250	55 (94) 150
A lateral line	113 (153) 188	130 (182.5) 250	38 (84) 175
Caudal peduncle	100 (134.4) 175	138 (156.3) 175	50 (75) 100
Caudal fin	88 (128) 163	25 (44) 63	Undifferentiated
Pectoral fin	50 (66.4) 88	25 (47) 75	Undifferentiated

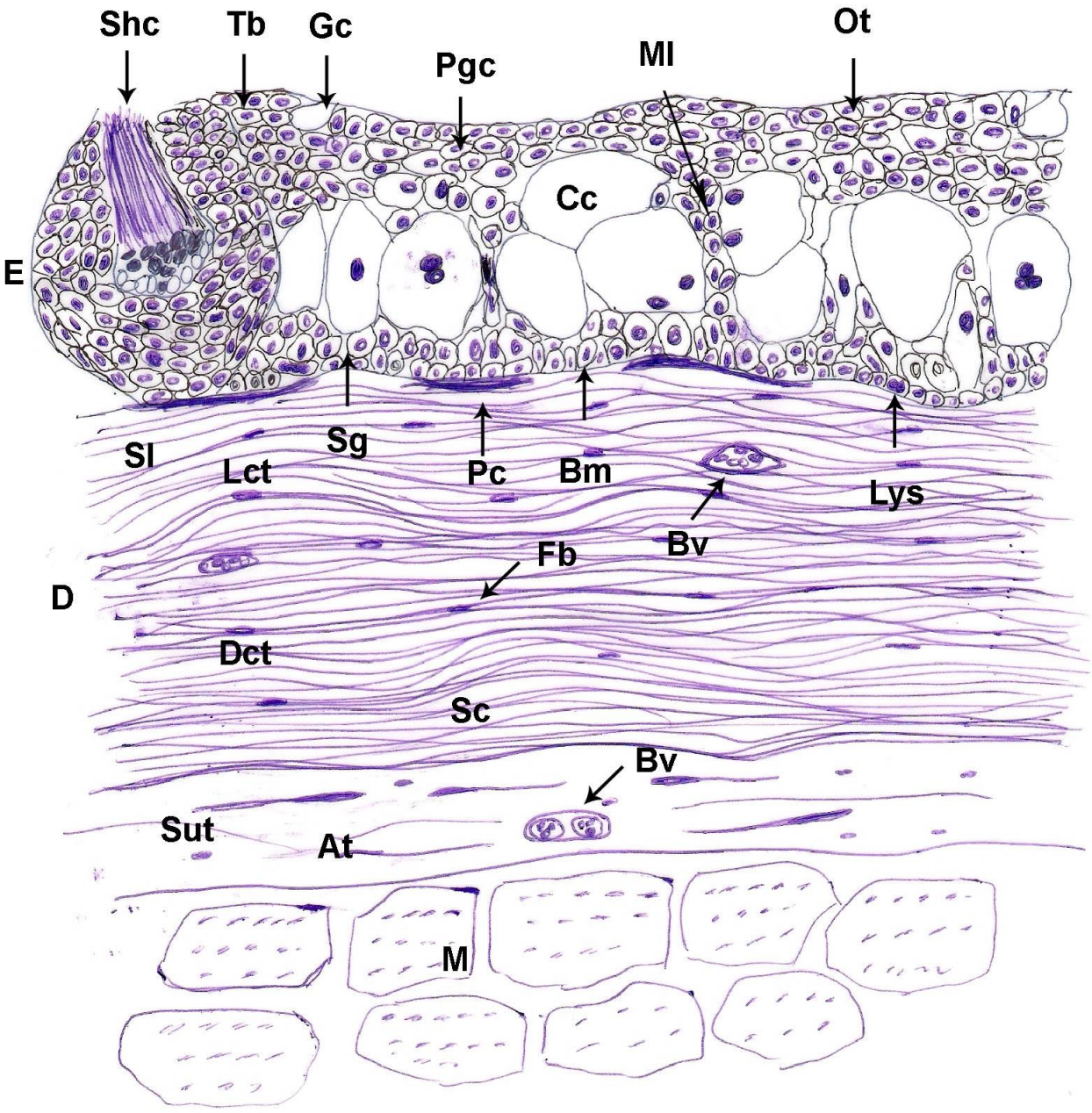

Figure (1). A diagramatic representation of the integument of *Mystus pelusius* showing its structure organization, (1000 x).

(At)Adipose tissue;(Bm) Basement membrane;(Bv) Blood vessels; (Cc)Club cell;(D) Dermis;(Dct) Dense connective tissue; (E)Epidermis; (Fb) Fibroblast; (Gc) Goblet cell; (Lct)Loose connective tissue; (Lys) LymphocytE; (M) Muscle; (Ml) Middel layer; (Ot) Outer layer; (Pc)Pigment cell; (Pgc)Polygonal cell; (Sc)Stratum compactum; (Shc)Sensory hair cell; (Sg)Stratum germinativum; (Sl) Stratum laxum; (Sut) Subcutis; (Tb) Taste bud.

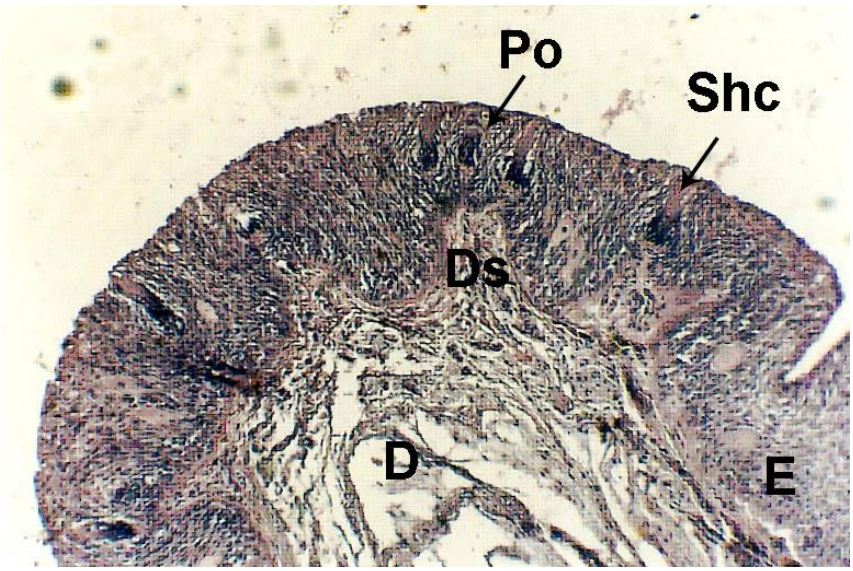

Figure (2). Vertical section of the integument of *Mystus pelusius* at lower lip region showing the general organization, papillary organ and dermal stalk, (PAS) (100X). (D) Dermis ,(Ds) Dermal stalk, (E) Epidermis ,(Po) Papillary organ (Shc) Sensory hair cell

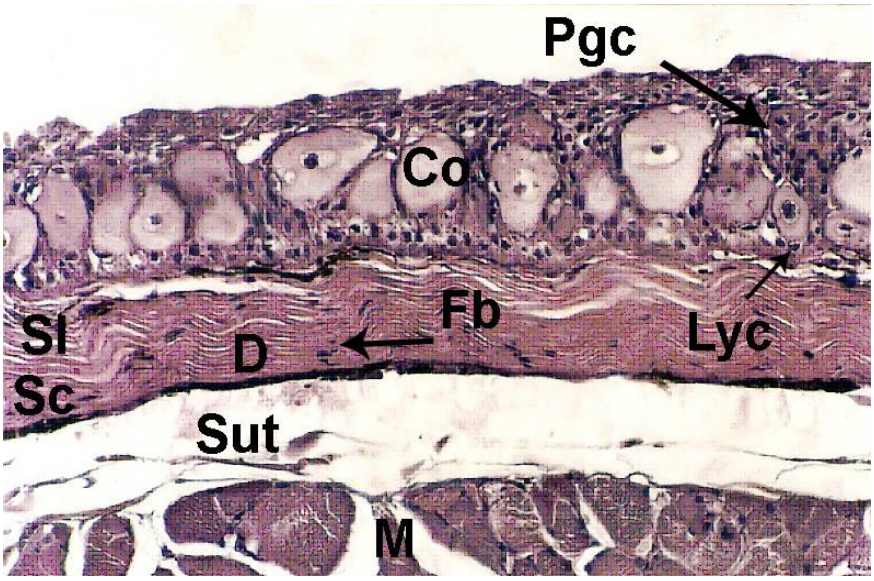

Figure (3). Vertical section of the integument of *Mystus pelusius* at head region showing the different integument layer and the types of cells , (HE) (200X).(Cc) Club cell, (D) Dermis,(Fb)Fibroblast,(Lys)Lymphatic space,(M)Muscle(Pgc)

Polygonal cell, (Sc)Stratum compactum, (Sl) Stratum laxum, (Sut) Subcut

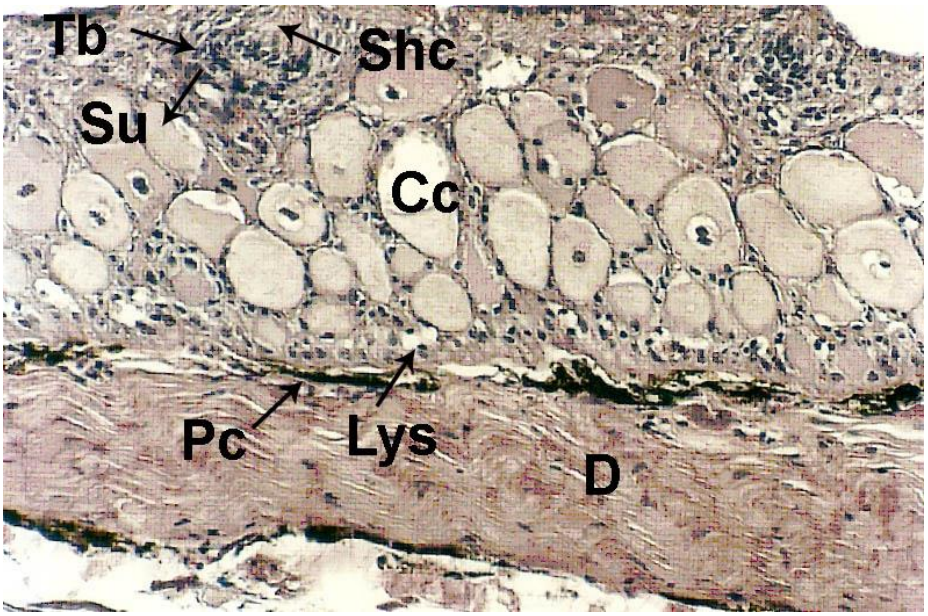

Figure (4). Vertical section of the integument of *Mystus pelusius* at dorsal region of the trunk showing the epidermis layers and the taste bud, (HE) (200X)).(Cc) Club cell, (D) Dermis ,(Lys)Lymphatic space,(Pc) pig mentcel ,(Shc) Sensory hair cell,(Su) Supporting cell (Tb) Taste bud

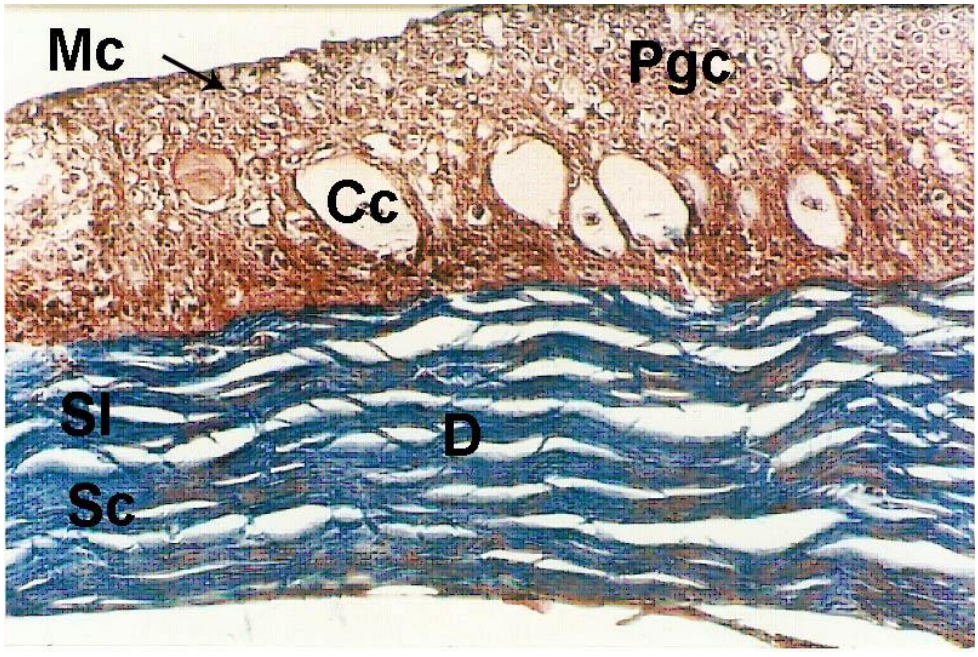

Figure (5). Vertical section of the integument of *Mystus pelusius* at caudal peduncle region showing the cellular layers of epidermis and arrangement of the dermal collagen fibers, (MTC)(200X).).(Cc) Club cell, (D) Dermis, ,(Pgc)Polygonal cell, (Sc)Stratum compactum, (Sl) Stratum laxum

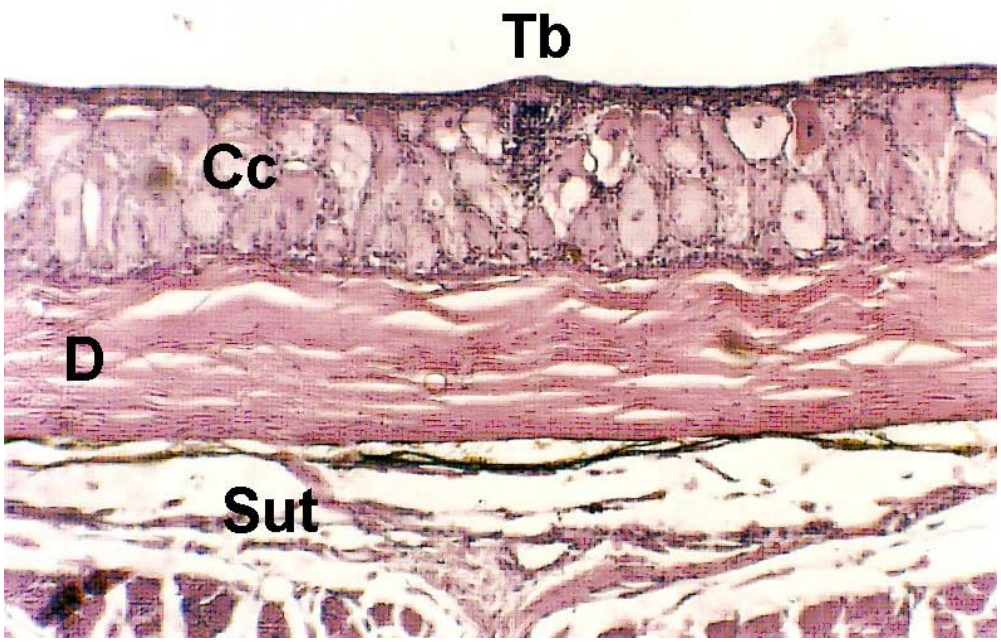

Figure (6). Vertical section of the integument of *Mystus pelusius* at ventral region of the trunk showing the integument layer, the position of subcutis layer, different types of epidermis cells and the position of taste bud,(HE)(100X). (Bv) Blood vessel,),(Cc) Club cell, (D) Dermis, (Sut) Subcutis, (Tb) Taste bud.

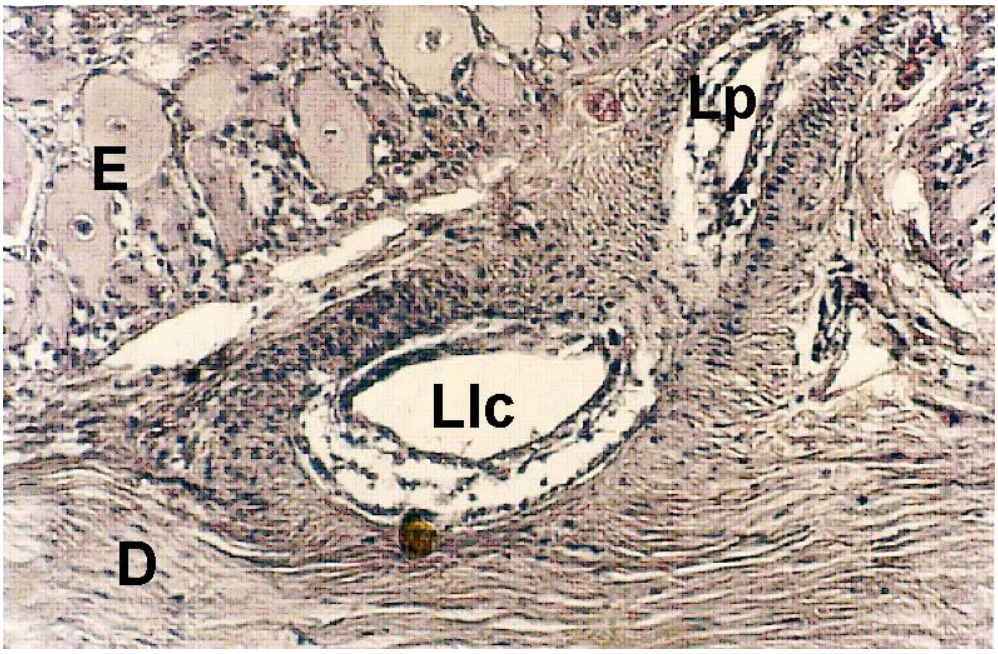

Figure (7). Vertical section of the integument of *Mystus pelusius* at lateral lin region showing the connection of the lateralis pore and the lateral line canal, ,(HE)(200X).(E) Epidermis , (D) Dermis, (Llc) Lateral line canal,(Lp) Lateralis pore.

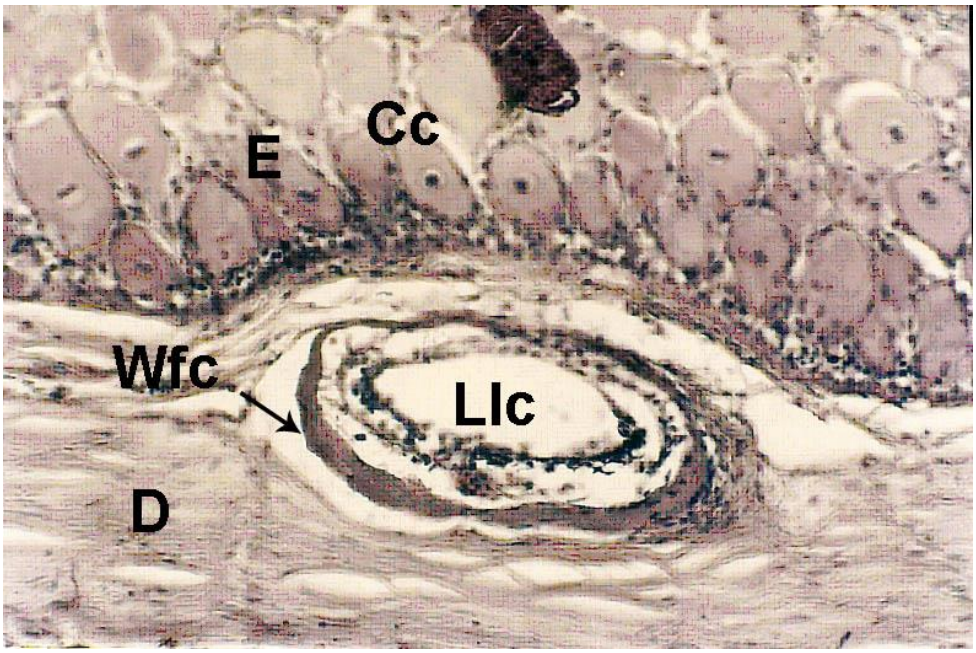

Figure (8) Vertical section of the integument of *Mystus pelusius* at lateral lin region showing the white fibrous cartilage which surrounded the lateral line canal , (HE)(200X). (c) Club cell. (D) Dermis, (E) Epidermis , (Llc) Lateral line canal, (Wfc) White fibrous cartilage.

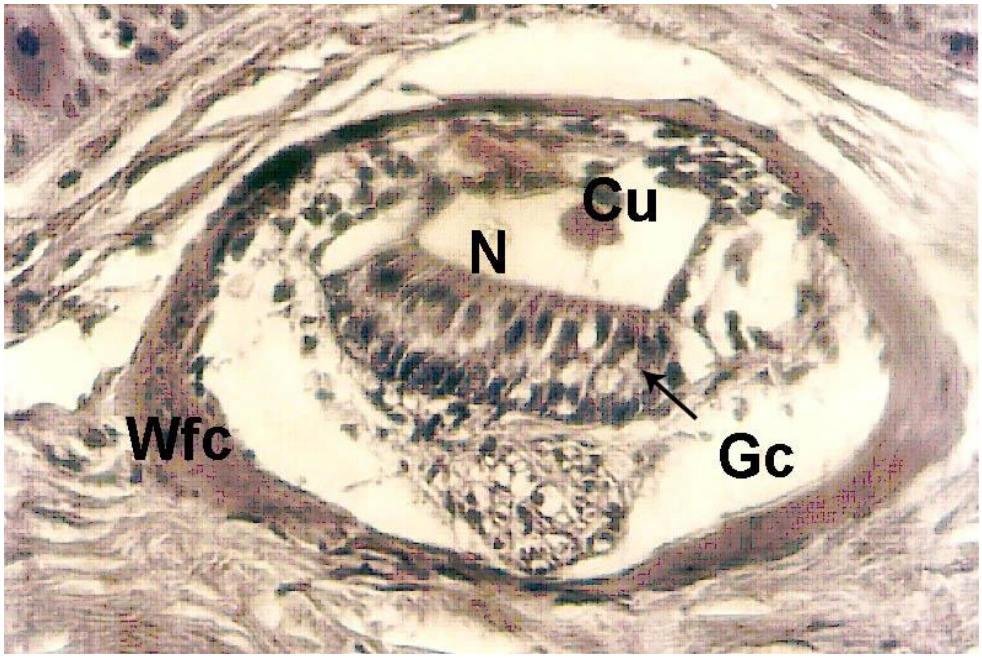

Figure (9) Vertical section of the integument of *Mystus pelusius* at lateral lin region showing the Neuromast and its cells and the cupula (HE) (400X).(Cu) Cupula ,(Gc) Goblet cell, (N) Neuromast , ,(Wfc) White fibrous cartilage.

Effect of exudates excreted by *Trichoderma harzianum* on the nitrogen fixation (C2H2- reduction) rate of the cyanobacteria *Anabaena variabilis*

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Abstract

Exudate of the fungus *Trichoderma harzianum* stimulate the nitrogen fixation (C2H2-reduction) of the cyanbacteria *Anabaena variabilis*. Autoclaving the exudates caused a decrease in the stimulation of the nitrogenase activity.Chemical analysis of the exudates showed it contain 95µg ml^{-1} protein, no free amino acids were detected, no nucleotides or nucleosides were found. Acid hydrolysis of the exudates showed the presence of bound amino acids and ammonia.

Low concentrations (one n mole ml^{-1}) of glutamic acid, aspartic acid, valine, glycine , and serine enhanced the nitrogenase activity and increased the heterocyst frequency of the cyanobacteria while threonine and proline seemed to reduce (inhibit) the nitrogenase activity at the same concentration.

دراسة تأثير افرازات الفطر *Trichoderma harzianum* على كفاءة تثبيت النتروجين الجوي (**C2H2-reduction**) للبكتريا الخضراء المزرقة ***Anabaena variabilis***

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الخلاصة

تمت دراسة تأثير افرازات الفطر *Trichoderma harzianum* على تنشيط عملية تثبيت النتروجين الجوي للبكتريا الخضراء المزرقة *Anabaena variabilis*_حيث وجد ان التركيز المخفف ١/٥٠٠٠ اعطى اعلى زيادة في فعالية أنزيم النتروجينيز (٢٠٠%) وزيادة في اعداد وتردد الخلايا المتخصصة بمعدل ٣٧% (Heterocyst) لاتمام عملية تثبيت النتروجين الجوي، فيما لم يكن هناك تأثير معنوي على زيادة في نمو البكتريا الخضراء المزرقة.ان دراسة تأثير الحرارة العالية (التعقيم الحراري الرطب) على مكونات الافرازات الفطرية وتحليل مكوناتها الكيمياوية بينت طبيعتها البروتينية حيث انخفض تأثيرها المحفز بنسبة ٦٠% ، ولم يظهر التحليل الكيمياوي وجود احماض امينية حرة او نيوكليوتيدات او نيوكليوسيدات ، وان التحليل الحامضي للافراز الفطري بين وجود احماض امينية مرتبطة اظافة الى الامونيا.

ان معاملةالمزارع البكتيرية بثمانية احماض امينية (تركيز ١ نانومول /سم^{3}) بصورة منفردة ادى الى زيادة معنوية في فعالية انزيم النتروجينيز وتردد الاجسام المتخصصة (Heterocyst) من قبل الاحماض الامينية الاتية: حامض الكلوتاميك، حامض الاسبارتيك، فالين، الكلايسين، السيرين و الالينين، في حين كان لحامضي الثريونين والبرولين تأثير مثبط ولنفس التركيز.

Introduction

In an earlier communication it was reported that the fungi *Trichoderma*

harzianum Rifai and *Aspergillus flavus* link ex.Fries affect the nitrogen fixation and growth of the cyanobacteria *Anabaena variabilis* (kűtz). Stimulatory as well as inhibitory agent were released by the fungi , and the stimulating agents could be separated by dialysis from the fungal medium and were thus found to be of higher molecular weight than the inhibiting agents (1).

Exudates from the fungus *Sclerotinia sclerotiorum* (Lib) De Bary have been throughly investigated and were found to contain proteinaceous compounds , free amino acid ,ammonia and enzymes (2,3). Later (4) found soluable carbohydrates and different salts in the exudates excreted by the fungus *Fusarium culmorum* (W.G.Smith) Saccardo.

In this study exudates excreted by *Trichoderma harzianum* were collected and tested with regard to their influence on the nitrogen fixation rate of the cyanobacteria *Anabaena variabilis*.

Material and Methods

Microorganisms and culture condition:

The cyanobacteria *Anabaena variabilis* 1403/12 (Cambridge culture collection of algae & protozoa) was grown axenically on modified nitrogen –free ASM medium (5)supplemented with trace elements (6).The cyanobacteria was incubated at 26c˚ and 62 µEm $^{-2}$ S $^{-1}$.The fungus *Trichoderma harzianum rifai* ,was grown for 14 days on malt extract agar (difco),in the dark at 28c˚. All further experiments were conducted at 26c˚ and 62 µEm $^{-2}$ S $^{-1}$.

Collection of fungal exudates:

When *T. harzianum* had grown for 14 days on malt extract slants, exudates had accumulated as yellowish watery droplets on the fungal surface . Such exudates were collected with Pasteur pipettes , and were then diluted with distilled water up to 5 or 10 ml using sterile conditions . The diluted exudates was then passed through Sartorius Millipore filters (0.2µm) in order to remove the fungal spores which might have adhered to the pipettes during the collection of the exudates ,1ml of 10 times diluted fungal exudate was analysed for free and combined amino acid contents .The samples were hydrolyzed for 24 hours by 6 N HCL, and the concentrations of the amino acids determined using Durrum D500 amino acid analyser.

Cyanobacteria nitrogenase activity (C2H2-reduction):

The nitrogenase activity was determined according to the method of Stewart *et al.* (7,8) . Acetylene gas equal to 10% of the total volume of the incubation flask was injected . After one hour , one ml gas phase samples were withdrawn and analyzed for

concentration of ethylene produced using Perkin – Elmer 880 gas chromatograph fitted with a Porapak T (50-80 mesh) , column run at 100c˚ . The flasks contained were aerated before and after each nitroginase activity measurement . Experiments were run accordingly , testing:

a) Effects of fungal exudates on cyanobacterial nitrogenase activity:

Effects of the exudates were studied by incubating 0.5 ml of an axenic cyanobacterial culture in 7ml , sterile glass serum bottles containing one ml ASM medium . 0.5 ml of sterile diluted fungal exudates being untreated or autoclaved , was added in a separate series . Nitrogenase activity (C2H2-reduction) was measured every 24 hours.

b) Effects of amino acids on nitrogenase activity , growth and heterocyst frequency:

Aliquots of an axenic cyonobacterial culture suspension were diluted to one n mole/ ml as a final concentration of the following amino acids:aspartic acid , threonine, serine , glutamic acid, proline , glycine, alanine and valine. The experiment was carried out in 70ml Erlemyer flasks containing 10 ml of cyanobacterial suspension . Nitrogenase activity was measured every 24hours ,chlorophyll a concentration ,heterocyst frequency was determined after 72hours.

Results & Discussion

Diluted fungal exudates had stimulatory effects on the nitrogen fixation (C2H2-reduction) rate of the cyanobacteria studied (Fig.1). Maximum effects was after 72 hours of treatment . The sterile , nonautoclaved exudates increase the nitrogenase activity by 200% comparing with the untreated control while the autoclaved exudates increase the activity rate by 40% ,this result indicate presence of more than one stimulatory factors in the fungal exudates , one is proteniaceous in nature, while the more effective once is the nonproteinaceous, Colotelo. *et al*. 1971(2) has characterized the chemical constiluents of exudates secreted by the fungus *S. sclerotiorum*.

The chemical analysis of the exudates shows that the total protein content was 95µg ml^{-1} , no free amino acids were detected . Acid hydrolysis of the exudates for 24hours showed presence of bound amino acids (table 1) and 1.25 µ moles ml^{-1} bound ammonia . These results partly agree with (2) and (8) who found free and bound amino acids and free ammonia in the exudates of *S. sclerotiorum* .Paper chromatography of the exudates indicated no nucleotides and nucleosides when examined

visually under an ultraviolet irradiant lamp according to the method of Bednar (9).

The fungal exudates had to be diluted before maximum effects on nitrogen fixation could be obtained. Out of the concentration tested , the dilution of 1:5000 gave the highest increase in nitrogen fixation , 50% higher than the control after 72 hours of treatment ,also the heterocyst frequency was increased by 44% (unpublished data).

Treatment of the cyanobacterial culture *A. variabilis* with one n mole ml^{-1} of eight amino acids seperatly ,which was found in the hydrolyzed exudates of the fungus *T. harzianum* showed that glutamic acid, aspartic acid ,valine, glycine, serine and alanine increase the heterocyst frequency and nitrogen fixation (Table 2), wherase proline and threonine acted as inhibitors.

No significant effect on growth of cyanobacteria at the end of the experiment was observed (Table 2) . A conclusion derived that low concentration of some kind of amino acids can act as stimulatory agents for nitrogen fixation of cyanobacteria .The above result confirmed the correlation between the nitrogen fixation and heterocyst frequency which has been shown in this association between *A. variabilis* and *T. harzianum* (1) . It is possible that the fungal exudates may serve as an inducer for heterocyst initation by breaking down the inhibitory zome described by (10) and (11), thus leading to an increase in heterocyst frequency and nitrogenase activity.

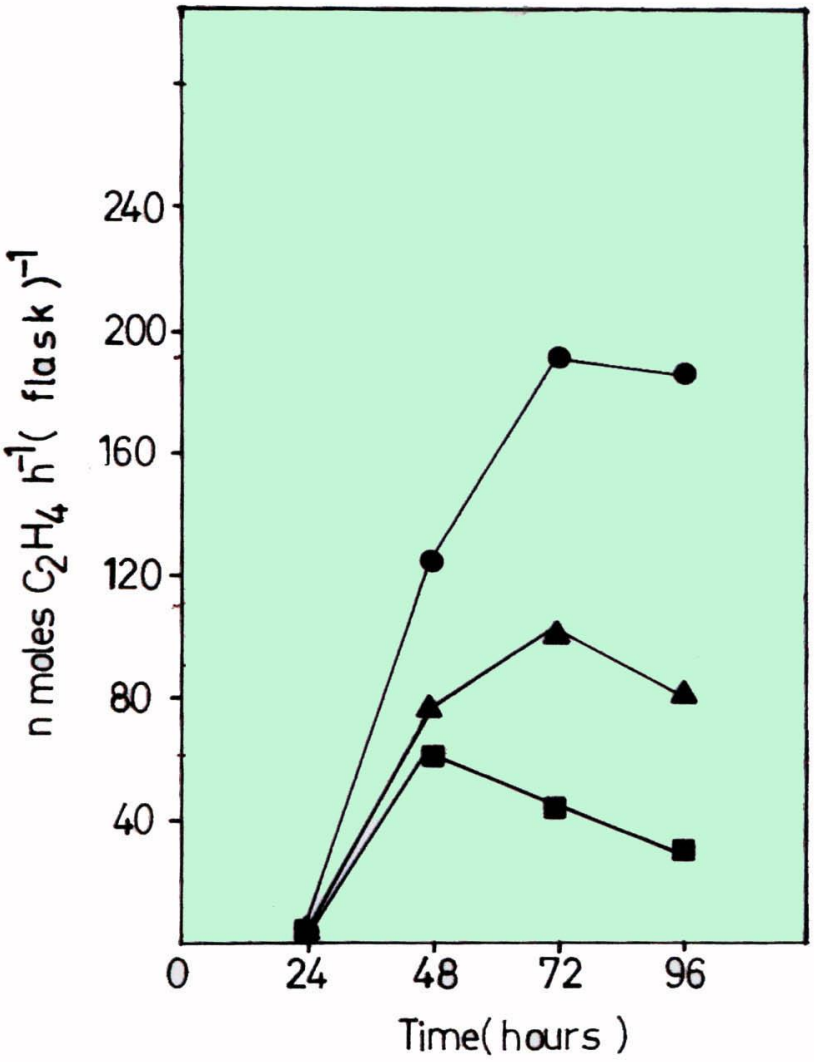

Fig. 1. Effect of the exudate excreted by the fungus *T. harzianum* on the nitrogen fixation (C_2H_2-reduction) of the cyanobacteria *A.variabilis*. ■—■ control, ●—● sterile filtered exudate, ▲—▲ autoclaved exudate.

Table 1 : Amino acids found in the hydrolyzed exudates excreted by the fungus *T. harzianum*

Amino acids	n moles ml^{-1}
Aspartic acid	96
Threonine	75
Serine	101
Glutamic acid	109
Proline	65
Glycine	112
Alanine	103
Valine	53
Methionine	7
Isolucine	30
Lucine	39
tyrosine	13
Phenylalanine	19
Histidine	12
Lysine	21
Arginine	12
Total	867 n moles

Total ammonia concentration =1250 n moles ml^{-1}

Table 2 : Nitrogen fixation (C2H2- reduction) , heterocyst frequency and growth of the cyanobacteria *A. variabilis* treated with one n mole of 8 amino acids separately

Amino acids	* µg Chlor. a ml^{-1}	** % of heterocyst	*** N-ase activity n moles h^{-1}/ µg Chlor. a
Control	5.78	4.6	8.02
Glutamic acid	6.14	8.0	17.40
Aspartic acid	5.79	7.2	10.28
Serine	5.69	5.8	14.24
Threonine	5.81	5.6	6.18
Valine	6.06	6.6	15.54
Glycine	5.78	6.8	10.48
Alanine	5.87	5.5	9.02
Proline	5.85	5.0	5.72

* the result are of three replicates for each treatment and after 160 hours of starting the experiment.

** Heterocyst are percentage of 1000 counted cells.

*** Maximum nitrogenase activity after 41-65 hours of treatment.

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Heavy metal contamination of drinking water in the city of Baiji

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Abstract

In order to ascertain water quality for human consumption and determined the sources of contamination, heavy metal were evaluated in the drinking water supplies to the city of Baji and its surrounding villages in Iraq. Standard methods were used for determining the concentration of heavy metals include Pb , Cd, Cu, and Zn in drinking water samples by atomic absorption spectrophotometer supplied with a carbon rode atomizer to increase the sensitivity. The results showed that the amount of lead present in the drinking water is high and ranges from 0.06± 0.03 to 0.14± 0.02 ppm , cadmium value were from the below detection limit to 0.01 ppm, copper value were ranges from 0.21± 0.08 to 0.58± 0.04 and zinc value were ranges from 1.57 ±0.37 to 2.30±o.45. to investigate the sources of contamination a samples from waste water of Baiji refineries and Baji power station have been taken to measures Pb , Cd, Cu, and Zn contents. No correlations were found between metal concentrations in the drinking water samples. This means that the contamination might come from corrosion of the pipes line system

Keywords: heavy metal ions, drinking waters , water contamination

تلوث مياه الشرب بالعناصر الثقيلة في مدينة بيجي

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الخلاصة

لكي نتحقق من نوعية الماء الصالحة للاستهلاك الإنساني ، وإيجاد مصادر التلوث بالمعادن الثقيلة، تم تقييم الماء المجهز للشرب في مدينة بيجي والقرى المحيطة بها، وقد استخدمت الطرق القياسية لتقرير تركيز المعادن الثقيلة والتي تتضمن : الرصاص، الكادميوم، النحاس والزنك في عينات الماء الصالح للشرب باستخدام تقنية الامتصاص الذري Spectrophotometer مجهز بقطب كربون لزيادة الحساسية ، وقد أظهرت الفحوصات النتائج التالية:

- كمية الرصاص بمجاميع تتراوح من $(0.06 \longrightarrow 0.14)\ PPm$
- قيمة الكادميوم تراوحت من تحت حد الكشف إلى $0.01\ PPm$
- قيمة النحاس تراوحت مابين $(0.21 \longrightarrow 0.58)\ PPm$
- قيمة الخارصين كانت مجاميع من $(0.37 \longrightarrow 1.57)\ PPm$

لتحري مصادر التلوث أخذت عينات من مياه الصرف الصحي ومن تبريد مصافي بيجي وتبريد محطة الطاقة الكهربائية في بيجي وتم قياس تراكيز الرصاص، الكادميوم والنحاس فيها ولم تثبت أية علاقة ما بينها وبين المياه المجهزة للمدينة وهذا يؤيد بأن العناصر الثقيلة الموجودة في مياه الشرب قد يكون سببها تآكل شبكة المياه.

Introduction:

The presence of toxic metals in human body is dangerous causing

serious health problems through interfering with normal biological function.

Some heavy metals have been reported to be of bio-importance to human and others like As, Cd, Pb, and methylated forms of Hg have been reported to have no known biological function in human and consumption even at very low concentrations can be toxic [1].

The widespread of contamination with heavy metal in the last decades has raised public and scientific interest due to their dangerous effects on human health This has led researchers to study the pollution with heavy metal in water and to determine their permissibility for human consumption.

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Heavy metals include lead, cadmium, cobalt, zinc, arsenic, mercury, silver, chromium, copper, iron, and platinum.[2].

Heavy metals concentration in the water cannot be attributed to geological factors alone, but human activities do modify considerably the mineral composition of water. The recent population and industrial growth has led to increasing production of domestic, municipal and industrial wastes. Heavy metals are natural components and cannot be degraded or destroyed. to a small extent, they enter our bodies via food, drinking water and air[3].

Along with many other toxic compounds in the environment, a lot of heavy metals, or the metals with a very high density, are present in our immediate environment. These heavy metals may contaminate water supplies, the natural water analysis for physical, chemical properties including trace element contents are very important for public health studies [4].

The biotoxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the bio-recommended limits. Heavy metals disrupt metabolic function in two basic ways:

- First, they accumulate and thereby disrupt function in vital organs and glands such as the heart, brain, kidneys, bone and liver.
- Second, they displace vital nutritional minerals from where they should be in the

body to provide biological function [5].

Individual metals exhibit specific signs of their toxicity, the following have been reported as general signs associated with lead, cadmium, arsenic, and zinc poisoning.

Lead is the most significant toxin and if released into the environment can bio accumulate and enter the food chain. At relatively low levels of exposure, these effects may include interference in red blood cell chemistry, and it interferes with normal cellular metabolism. Lead has damaging effects on body nervous system. acute and chronic damage to the central nervous system (CNS) and peripheral nervous system (PNS) .Lead affects children by leading to the poor development of the grey matter of the brain,

Materials that contain lead have frequently been used in the construction of water supply distribution systems and plumbing systems in private homes and other buildings. The most commonly found materials include service lines, pipes, brass, bronze fixtures, solders and fluxes. Lead in these materials can contaminate drinking water as a result of the corrosion that takes place when water comes into contact with those materials [6].

Cadmium, a metal, is found naturally in very low concentrations in most rocks, as well as in coal and petroleum and often in combination with zinc. Cadmium uses include electroplating, nickel-cadmium batteries, paint and pigments, and plastic stabilizers. It is introduced into the environment from mining and smelting operations and industrial operations, including electroplating, reprocessing cadmium scrap, and incineration of cadmium-containing plastics. Cadmium may enter drinking water as a result of corrosion of galvanized pipe [7].

Cadmium is toxic at extremely low levels. In humans, long term exposure results in renal dysfunction, characterized by tubular proteinuria. The kidney is considered to be the critical target organ in humans chronically exposed to cadmium by ingestion.

High exposure can lead to obstructive lung disease, cadmium pneumonitis, resulting from inhaled dusts and fumes. Cadmium is also associated with bone defects, osteomalacia, osteoporosis and spontaneous fractures, increased

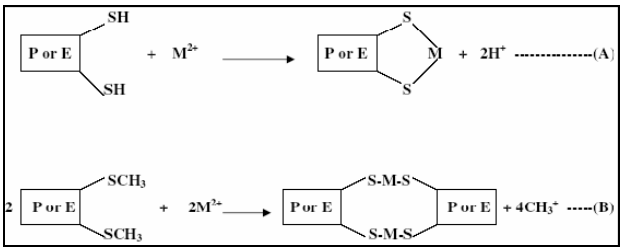
blood pressure and myocardic dysfunctions[8].

Copper, a reddish-brown metal, is often used in plumbing of residential and commercial structures that are connected to water distribution systems. Copper contaminating drinking water as a corrosion by-product occurs as the result of the corrosion of copper pipes that remain in contact with water for a prolonged period. Copper is an essential nutrient, but at high doses has been shown to cause stomach and intestinal distress, liver and kidney damage, and anemia [9].

Zinc is considered to be relatively non-toxic, especially if taken orally. Excess amount can cause system dysfunctions that result in impairment of growth and reproduction The clinical signs of zinc toxicities have been reported as vomiting, diarrhea [10].

Heavy metal poisoning:

The poisoning effects of heavy metals are due to their interference with the normal metabolic processes, in the acid medium of the stomach, they are converted to their stable oxidation states (Zn2+, Pb2+, Cd2+,Cu2+) and combine with the molecules such as proteins and enzymes to form strong and stable chemical bonds. The equations below show their reactions during bond formation with the sulphydryl groups of cysteine -SH and sulphur atoms of methionine $-SCH_3$[11].



Where: P = protein; E = Enzyme; M = Metal

The hydrogen atoms or the metal groups are replaced by the poisoning metal and the enzyme is thus inhibited from functioning, whereas the protein–metal compound acts as a substrate and reacts with a metabolic enzyme. [12]. the deleterious effects of heavy metal ions have been attributed to their interactions with specific, particularly susceptible native proteins. Proved to inhibit very efficiently the spontaneous refolding of chemically denatured proteins by forming high-affinity complexes with thiol and other functional groups.

Therefore, the metal remains embedded in the tissue as metallo-

enzyme and can be conveniently replaced by another metal ion of similar size. Thus Cd2+ can replace Zn2+ in some dehydrogenating enzymes, leading to cadmium toxicity [13].

The most toxic forms of these metals in their ionic species are the most stable oxidation states as will as heavy metals in the body multiply free radicals chain reactions several thousands, free radicals process contribute to the uncontrolled chain reaction causes several damages within the cells[14].

Material and method:

Prior to analysis all instruments were calibrated according to manufacturer's recommendation

The drinking and waste water samples were collected in pre-washed (with detergent, doubly de-ionized distilled water, diluted HNO3 and doubly de-ionized distilled water, respectively) polyethylene bottles from 5 drinking water stations in bajii, waste water of Baiji refineries, Baiji power station are taken in Dec. 2007.

One liter of each water sample was taken in duplicate at two deferent sampling periods approximately one month a part. the drinking water sample were obtained directly from the water pump after allowing the water to run at least 20 minutes while waste water samples were obtained from waste water pools [15].. These samples were analyzed by flameless atomic absorption spectrometry (Spectroil M Analysis Spectrometer) in triplicate to determine lead, cadmium, zinc and copper. Table (1) showed wave lengths that used to measure the concentration of these metals. A standard solution for each element under investigation was prepared and used for calibration [16].standards and samples are in disposable plastic sample holders and it is important that the sample holder be filled level with the top. The bottom of the disc electrode should be immersed in the sample before analysis [17]..

Result:

The results of heavy metal contents in drinking water are shown in Table 2. The results showed that all drinking water samples have higher concentration of lead than that recommended by WHO 1984.

.where it ranges: 0.06± 0.03, 0.14± 0.02 in station no. 4 and 2 respectively. The concentration of lead in waste water is too high

ranges: 0.76 in refinery and 0.64 in power station.

As shown in Table 2, the concentrations of cadmium of all drinking water samples under investigation were under the maximum permissible concentration of cadmium (FAO/WHO 1984). While the cadmium concentrations in waste water were 0.07 in refinery and 0.03 in power stations.

This study show that the zinc content in water samples less than the maximum limit allowed of zinc for drinking water. It was range of 1.57± 0.37 to 2.30 ppm, the lowest and highest values were in station 1 and 3 respectively. Also there is some variation between stations. Zinc concentrations in waste water were 8.3 in refinery and 5.8 in power station.

Copper concentrations in the drinking water samples were in the range of 0.21± 0.08 , 0.58± 0.04 ppm. The lowest and highest values were in station 4 and 5 respectively, but even in Station 5 Cu was considerably below the limit of 1.0 mg/1 permitted by WHO in drinking water. Waste water contained of copper was 1.2 in refinery and 1.8 in power station.

Discussion:

The data obtained from the study recalled that drinking water have a high level of lead than that permitted by WHO. The study is in disagreement with Soylak (2002) who was found that the lead level in Tigris river below the permissible limit given by WHO.

This study showed that the river contamination occur in Iraqi area might be due to release a large amount of petrol due to several explosions of main pipe line close to the city of Baji, also our study showed that the waste product of Baji refineries and Baji power station which release directly to the river stream have a high level of lead as shown in table 3 in addition to that some station still using lead pipe which increase the level of lead in drinking water, lead can be found in the solder used to join copper pipes, and a major source of environmental Pb, particularly in urban areas, is due to the combustion of leaded petrol. Lead is discharged by vehicles into air, and then adsorbed from the air by environmental samples such as soil and plant than inter the water ways from soil. The cadmium was blow the normal level in this study.

The study showed that the zinc level in drinking water samples

were with in normal in spite of the presence with high amount in waste water of Baiji refinery and power station.

The study showed that the concentration of copper with in normal except that of waste water which are above normal.

Our study is in disagreement with Kalid (2006) who found that the lead level in the Salamani drinking water below the accepted internationality level. Further studies are recommended to study the sources of contamination of the drinking water with heavy metal and investigate other sources of contamination

Although heavy metals cannot be avoided due to their prevalence in the environment, several measures besides avoidance of the source of heavy metals can be taken to treat toxicity.

Diet, nutritional supplements and chelating agents such as DMSA are safe and effective means to reduce heavy metal toxicity. No correlations were found between metal concentrations in the drinking water samples.

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Table 1. heavy metal contents n drinking water by the World Health Organization (WHO) and Wavelength of absorption in atomic absorption spectrophotometer.

Heavy metal	Max. acceptable conc. (WHO)	Wavelength (nm)
Lead	0.01 mg/L	283
Cadmium	0.003 mg/L	228
Zinc	5.0 mg/L	213
copper	1.0 mg/L	324

Table 2. concentration of heavy metal in different water samples.

Station no. (Drinking water)	Lead (Pd) mg/L	Cadmium (Cd) mg/L	Zinc (Zn) mg/L	Copper (Cu) mg/L
Station 1	0.12 ± 0.04	0.01	1.57 ± 0.37	0.38 ± 0.09
Station 2	0.14 ± 0.02	0.00	1.80 ± 0.21	0.32 ± 0.12
Staton 3	0.08 ± 0.02	0.00	2.30 ± 0.45	0.43 ± 0.9
Station 4	0.06 ± 0.03	0.01	2.10 ± 0.18	0.21 ± 0.08
Station 5	0.13 ± 0.06	0.00	1.65 ± 0.32	0.58 ± 0.04

Table 3. concentration of heavy metal in waste water samples.

Waste water	Lead (Pd) mg/L	Cadmium (Cd) mg/L	Zinc (Zn) mg/L	Copper Cu) mg/L
Baiji refineries	0.76 ± 0.12	0.07 ± 0.02	8.3 ± 2.4	1.2 ± 0.1
Baiji power station	0.64 ± 0.08	0.03 ± 0.01	5.8 ± 0.8	1.8 ± 0.3

TEXT-INDEPENDENT SPEAKER IDENTIFICATION

SYSTEM USING PROBABLISTIC NEURAL NETWORK

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ABSTRACT

Text-independent closed set speaker identification system is achieved using a Probabilistic Neural Network (PNN) as a classifier and Reflection Coefficients(RC) as a speaker feature. The system is evaluated with a database consisting of 28 speakers(21 male and 7 female). Each speaker has three totally different sentences, the first is used for training and the rest are considered as a testing sentences. The system correctly identified all the database speakers when tested with noise free speech for the two test sentences. For 30 and 20 dB SNR noisy speech, the performance is almost unchanged.

Keywords: Artificial Neural Network (ANN), Automatic Speaker Recognition (ASR), Cepstral Analysis, Probabilistic Neural Network (PNN), Speech Processing, Voice Biometric.

الملخص

يقدم هذا البحث محاكاة لنظام تعريف المتكلم غير المعتمد على النص و من النوع المغلق. أستعمل النظام معاملات الانعكاس (RC) كمعلم للمتكلّمِ ، والمصنف كَانَ شبكة عصبيةَ احتمالية (PNN). قُيّمَ النظام بقاعدةِ بيانات تشْملُ ٢٨ متكلمِ (٢١ ذكر و٧ إناث). قرأ كُلّ متكلّم ثلاثة جُمَلِ مختلفةِ كلياً، الأولى استعملت للتدريب والبقيةِ اعتبرت جُمَل اختبار. تمكن النظام من تعريف جميع المتكلمين في قاعدةِ البيانات بصورة صحيحة عندما أختبر بالكلام النقي لجملتي الاختبار. ل٣٠، ٢٠ dB كان أداء النظام مقارب للنتيجة السابقة.

I. INTRODUCTION

Automatic Speaker Recognition(ASR) (also called Voice Biometric)is the use of a machine to extract, characterize and recognize the information

about speaker identity from a spoken phrase[1]. Banking by telephone, telephone shopping, database access services, information services, security control for confidential information areas, and remote access to computers are applications of Automatic Speaker Recognition. Speaker recognition has two branches: identification and verification. Speaker verification task is to verify the claimed identity of person from his voice. In speaker identification there is no identity claim and the system decides who the speaking person is [2]. Open-set speaker identification decides to whom of the registered speakers unknown speech sample belongs or makes a conclusion that the speech sample is unknown. Closed-set speaker identification decides to whom of the registered speakers unknown speech sample belongs. Depending on the algorithm used for the identification, the task can also be divided into text-dependent and text-independent identification. The difference is that in the first case the system knows the text spoken by the person while in the second case the system must be able to recognize the speaker from any text. The process of speaker identification is divided into two main phases: speaker enrollment and identification phase. Both phases include the same first step, feature extraction. The variations between speakers is called inter-speaker variations and the term intra-speaker represents variations for the same speaker. For speaker recognition, features that exhibits high speaker discrimination power, high inter-speaker variability , and low intra-speaker variability are desired[3]. 1978 Larry L. Pfeifer utilized vowel sound as a basis for extracting speaker characteristic and developed a method of text-independent speaker identification system, and a rate of 95 percent of correct identified speaker (from a database of 20 speakers, 10 male and 10 female) . Twelve reflection coefficients were used as a feature [4]. In 1999 Stephen A. Zahorian described a new neural network algorithm for speaker identification with large groups of speakers. The technique was derived from a technique in which an N-way speaker identification task is partitioned into N*(N-1)/2 two-way classification tasks. In that new approach, two-way neural network classifiers, each of which is trained only to separate two speakers, are also used to separate other pairs of speakers. High recognition rate was gained from that network[٥]. In 2003 Mustafa Sarimollaoglu, Serhan Dagtas, Kamran Iqbal and Coskun Bayrak developed a text-independent speaker identification system based on Probabilistic Neural Network (PNN). PNNs supply flexibility and straightforward design make the system easily operable along with the successful classification results. The system

was able to correctly identify 96% of the speakers, using 0.8 seconds of test samples from each speaker. 20 Mel-scaled cepstral coefficients (excluding 0th) were used as a feature vector. The database consists of speech samples from 28 adults, 21 male and 7 female[٦].

Conclusive model of speaker identification system is shown in Figure (1).

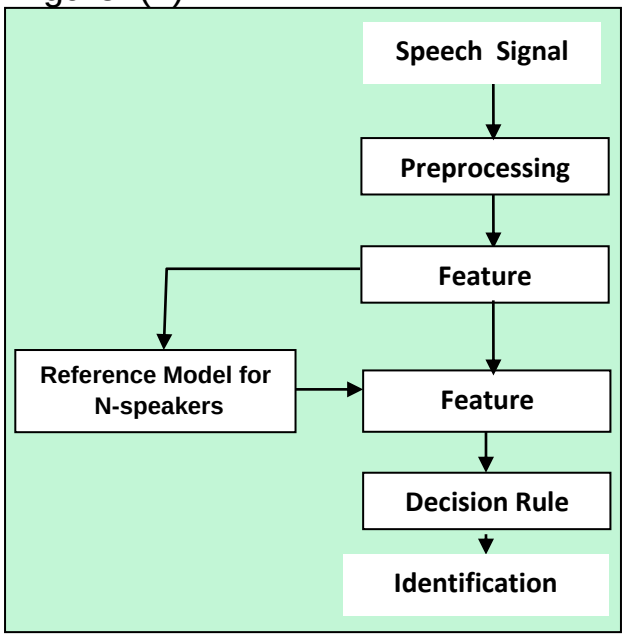

Figure (1) Speaker Identification System

The aim of feature Extraction is to transform raw speech signal into a compact but effective representation that is more stable and discriminative than the original signal. Feature Matching involves the actual procedure to identify the unknown speaker by comparing extracted features from his/her voice input with the one from a set of known speakers. One of the approaches used is the Probabilistic Neural Network (PNN) [1].

II. SPEECH PROCESSING TECHNIQUES

Since the speech signal is a slowly varying signal or a "quasi-stationary" when examined over a sufficiently short period of time (20-30 milliseconds)[7], this leads to the useful concept of describing human speech signal, called "short-term analysis", where only a portion of the signal(frame) is used to extract signal features one at a time.

Speech production can be modeled by the so-called "source-filter" model. The voice source is either a periodic pulse stream or uncorrelated white noise, or a combination of these[1].

It can be noted that speech signal is predicted as a linear combination of the previous *p* samples. Therefore, the speech production model is often called linear prediction (LP) model or the autoregressive model.

Before the system calculates the features, the speech signal is segmented(with each segment lasts for 20-30 milliseconds) [7] into overlapping(with 20-50% from the frame length) (to ensure that the samples in the last period in previous segment, will not be lost or attenuated very much and, therefore uncounted) frames. Theses frames are windowed through a appropriate window like "Hamming Window"[8]. Then the

needed techniques are applied resulting in coefficients (each coefficients vector resulting from

only one frame) like Linear Prediction Coefficients(LPC), Reflection Coefficients(RC) , Linear Prediction Cepstral Coefficients(LPCC) and Mel-Frequency Cepstral Coefficients (MFCC)….etc.

When the vocal tract is modeled with the lossless tube model[7], at each tube junction, part

of the wave is transmitted and the remainder is reflected back, The reflection coefficients are the percentage of the reflection at these discontinuities. If Levinson-Durbin's algorithm[9] is used to solve the LPC(Linear Prediction Coefficient) equations[1], the reflection coefficients κ_m are the intermediate variables in the recursion.

III. PROBABLISTIC NEURAL NETWORK(PNN)

Probabilistic Neural Network is one of many neural networks[10], their general use is in classification problems. Their advantage over other methods is flexibility and the straightforward design.

Training time which increases substantially with increasing population size is not a disadvantage for PNNs. The PNNs implement window estimator by using a mixture of Gaussian basis functions. If a PNN for classification in K classes(in speaker recognition K referred to the number of speakers) is considered, the probability density function $f_i(x_p)$ of each class k_i is defined by:

$$f_i(x_p) = \frac{1}{(2\pi)^{d/2}\sigma^d M_i}\sum_{j=1}^{M_i}\exp\left(-\frac{1}{2\sigma^2}(x_p - x_{ij})^T(x_p - x_{ij})\right), i = 1,2,....K.....(1)$$

where x_{ij} is the j-th training vector from class k_i, x_p is the p-th input vector, d is the dimension of the speech feature vectors, and M_i is

the number of training patterns in class k_i. Each training vector x_{ij} is assumed a centre of a kernel function, and consequently the number of pattern units in the first hidden layer of the neural network is given as a sum of the pattern units for all the classes. The

and competitive layer. In the first layer(the number of its neurons is Q), vector distances between the input vector p and each row of the input weight matrix IW1,1 are calculated and then multiplied by the bias b(which is a vector all its elements have the same value as given by Equation(3)with length Q). Activation function of the radial basis layer is given as:

$$radbas(n) = e^{-n^2} \quad \text{.....(2)}$$

radbas function produces its maximum of 1 where the input p is identical to w , and the output decreases as the distance between p and w increases.

Bias *b* allows the sensitivity to be adjusted and defined as:

$$b^1 = \frac{\sqrt{-\ln 0.5}}{\sigma} \quad \text{.....(3)}$$

The spread of radial basis function (σ) represents the typical variance σ^2 acts as a smoothing factor, which softens the surface defined by the multiple Gaussian functions.

The PNN used in the system is shown in Figure (2). This model has two layers: radial basis layer

distance between input vectors. Weights of the two layers (*IW*1,1 , *LW*2,1) are set to the matrices which are formed from the training vectors and the target vectors, respectively. The number of classes of input data, K, is equal to the number of users trained in the system. Q is the number of input/target pairs. Each target vector has K elements one of which is 1 and the others are 0. No training is neede ε construction of PNN in contrast to other neural networks. Input feature vectors are directly assigned as the weight matrix *IW*1,1. This property saves processing time for training beside more neurons are required. In the testing phase, feature vectors extracted from a test sample are entered to the PNN as inputs. In the presence of an input vector, distances between the input and the training vectors are calculated and multiplied by *b* to form n1. *radbas* function designates a number close to 1 where input is

close to a training vector. An input vector may be close to several training vectors, which causes a1 to have several elements close to 1. In the second layer, a1 is multiplied by the *LW*2,1. This operation sums the groups of elements which correspond to each of the K classes. Then, *compete* function (which assigns 1 for the largest value, and 0 for all the others) is applied. Finally, occurrence of 1 in the k^{th} row of the output a2 means that the input vector *most probably belongs* to the kth class i.e., the k^{th} speaker.

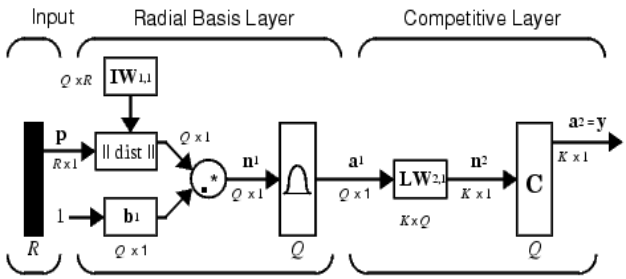

Figure (2) PNN Architecture and radbas function

IV. SIMULATION RESULTS

This section presents the results obtained from the simulation of the closed set text-independent speaker identification systems on personal computer. The simulation has been performed on the Pentium IV personal computer of 2.6 GHz CPU speed, using COOL edit pro.2000 as a speech editor and MATLAB software package (version 7) to simulate the system.

In this system, firstly the RC are calculated then used as an input features to PNN. The system is tested first with original speech data(noise free speech) and then an Additive White Gaussian Noise AWGN is added with signal-to-noise ratios(SNR)of 10,20 and 30 dB. PNN database consists of 28 speakers (21 males and 7 females). The number of speakers in the database is chosen in order to make the program execution time reasonable. All speakers read three sentences (one is used for training and the two others are for testing). The sentences were totally different. The recording is done by means of COOL edit software. Each sentence is of approximately 3-seconds long, and the recording is performed in normal room conditions with a sound card. The speech is sampled at 16 KHz with 16-bits/sample.

A. Simulation of the PNN System

The continuous speech signal is sectioned into frames of N samples with adjacent frames overlapping of L samples (L<N), The chosen values are N=320 samples(which is 20 ms) and L=120 samples.

A frame windowing is done using a Hamming window, and reflection coefficients(RC) are used as input to the network. Prediction order of RC was 18, 22, 26 and 30 and is denoted by "p".

To study the influence of number of frames applied to the PNN(i.e. the length of training and testing sentences)on the system, the number of frames are changed, as:
380,340,300,260,220,180,140,100 and 60. These number of frames are equal to: 4.7575, 4.2575, 3.7575, 3.2575, 2.7575, 2.2575, 1.7575 and 1.2575 seconds respectively. For cases where the number of required frames exceeds the sentence length, the same RC vectors were repeated to get the desired number. The same number of frames were used for training and testing.

During training, the RC vectors obtained from each speaker are used directly as an input weight matrix *IW*1,1, while the 1's in matrix *LW*2,1 are correctly positioned in correspondence with each speaker RC vectors .

During the testing, the RC vectors will be treated separately, i.e. each vector with length (p*1) is applied to PNN. The result of this vector is a number representing the speaker number.

When all the vectors from test utterance are applied, the system will count the number of times in which each speaker is appeared (for the present test utterance) and the speaker with largest number of appearance will be considered as the correct speaker .

The term "test 1" refers to the test with the second sentence in the database; and the term "test 2" refers to the test with the third sentence in the database. The system is trained with the 1st sentence.

Changing the order "p" and test sentences, the results for testing noise free speech for the PNN system are shown in Tables (1),(2),(3),(4),(5),(6),(7)and(8). The results for testing noisy speech for the PNN system(with p=30 and spread=0.1) are shown in Tables (9) ,(10) and (11).

Table(1)
Number of correct identified speakers for 28 speakers with noise free tested speech with p=30 and test 1

Number of Frames	Spread		
	0.05	0.1	0.15
380	28	28	28
340	28	28	28
300	28	28	28
260	28	28	28
220	28	28	28
180	28	28	28
140	28	28	28
100	25	25	24
60	16	14	15

Table(2)
Number of correct identified speakers for 28 speakers with noise free tested speech with p=30 and test 2

Number of Frames	Spread		
	0.05	0.1	0.15
380	28	28	27
340	28	28	27
300	28	28	27
260	28	28	27
220	27	28	28
180	28	28	28
140	27	28	26
100	21	21	21
60	18	17	15

Table(3)
Number of correct identified speakers for 28 speakers with noise free tested speech with p=26 and test 1

Number of Frames	Spread		
	0.05	0.1	0.15
380	28	28	28
340	28	28	28
300	28	28	28
260	28	28	28
220	28	28	28
180	28	28	28
140	27	27	27
100	24	25	24
60	14	19	18

Table(4)
Number of correct identified speakers for 28 speakers with noise free tested speech with p=26 and test 2

Number of Frames	Spread		
	0.05	0.1	0.15
380	27	27	27
340	27	27	27
300	27	28	26
260	27	28	28
220	27	27	27
180	27	28	27
140	27	27	27
100	24	24	21
60	21	20	19

Table(5)
Number of correct identified speakers for 28 speakers with noise free tested speech with p=22 and test 1

Number of Frames	Spread		
	0.05	0.1	0.15
380	28	28	28
340	28	28	28
300	28	28	28
260	28	28	28
220	28	28	28
180	28	27	27
140	27	27	27
100	25	25	24
60	16	15	18

Table(6)
Number of correct identified speakers for 28 speakers with noise free tested speech with p=22 and test 2

Number of Frames	Spread		
	0.05	0.1	0.15
380	27	28	27
340	27	28	25
300	27	28	25
260	27	28	26
220	27	28	27
180	26	27	27
140	26	26	27
100	22	23	20
60	18	17	16

Table(7)
Number of correct identified speakers for 28 speakers with noise free tested speech with p=18 and test 1

Number of Frames	Spread		
	0.05	0.1	0.15
380	28	28	28
340	28	28	27
300	28	28	27
260	28	28	28
220	28	28	28
180	28	28	27
140	26	27	27
100	23	24	24
60	14	15	15

Table(8)
Number of correct identified speakers for 28 speakers with noise free tested speech with p=18 and test 2

Number of Frames	Spread		
	0.05	0.1	0.15
380	27	27	25
340	27	28	25
300	27	27	26
260	27	28	26
220	27	27	26
180	26	27	25
140	25	24	25
100	21	22	22
60	22	20	19

Table(9)
Number of correct identified speakers for 28 speakers with noisy tested speech with SNR=10dB, p=30 and spread=0.1

Number of Frames	test1	test2
380	1	0
340	1	0
300	1	0
260	1	0
220	1	0
180	0	0
140	1	1
100	0	1
60	1	1

Table(10)
Number of correct identified speakers for 28 speakers with noisy tested speech with SNR=20dB, p=30 and spread=0.1

Number of Frames	test1	test2
380	26	26
340	26	24
300	25	23
260	26	25
220	26	25
180	26	23
140	24	19
100	20	17
60	11	14

Table(11)
Number of correct identified speakers for 28 speakers with noisy tested speech with SNR=30dB, p=30 and spread=0.1

Number of Frames	test1	test2
380	28	27
340	28	27
300	28	28
260	28	28
220	28	27
180	28	27
140	28	28
100	25	21
60	17	19

V. Conclusion

The PNN is an efficient method when used with the Reflection Coefficients. The number of frames taken from the speech signal is a crucial factor in the system performance, and a minimum of that number is required to make the system works properly. The prediction order is an important factor. For the PNN system used in this study, if the two previous factors are adjusted in a right manner, the network "spread" has a little impact on the system performance. Finally, the system performs well with SNR of 20 and 30 dB.

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Completeness of M-fuzzy metric spaces

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ABSTRACT

In this paper, the definition and basic concepts of M-fuzzy metric is introduced with some of its basic properties and some fundamental results concerning this concept, and then these aspects are used to introduce and prove the completeness of M-fuzzy metric spaces.

Part from M. Sc. thesis of first researcher

كمال الفضاءات المترية الضبابية باستخدام الدوال المترية M-

اماني التفات كاظم *

***قسم هندسة البرامجيات-**

كلية مدينة العلم الجامعة-بغداد-

العراق

فاضل صبحي فاضل **

****قسم الرياضيات وتطبيقات الحاسوب**

كلية العلوم-جامعة النهرين-بغداد-

العراق

الملخص

في هذه الورقة التعريف والمفاهيم الاساسية في الفضاءات المتريةالضبابية-M مقدم بالبعض من خواصه الاساسية وبعض النتائج الاساسية يتعلقان بهذه المفاهيم ثم هذه السمات تستعمل لتقديم واثبات كمال الفضاءات الضبابية باستخدام الدوال المترية-M

جزء من أطروحة الباحث الاول

Introduction

The concept of fuzzy sets was introduced by Zadeh in 1965 [1]. Since then, this concept is used in topology and some branches of analysis, many authors have expensively developed the theory of fuzzy sets and application.

We start with the obvious definition of fuzzy sets “A fuzzy set, termed by $\tilde{A}$, in a space of objects X is a class of events with a continuous grade of membership and is characterized by a membership function, termed as $\mu_{\tilde{A}}$, which associates for each x $\in$ X a real number in the interval [0, 1]”. The value of $\mu_{\tilde{A}}(x)$ represents of grade of membership of x in $\tilde{A}$, i.e., denotes the degree to which an element or event x may be a member of $\tilde{A}$ or belong to $\tilde{A}$, [2].

Chang, C. L. in 1968 used the fuzzy set theory for defining and introducing fuzzy topological spaces, while Wong, C. K. in 1973, discussed the covering properties of fuzzy topological spaces, [3].

Ercey, M. A. in 1979, studied fuzzy metric spaces and its connection with statistical metric spaces, Ming P. P. and Ming L. Y. in 1980, used fuzzy topology to define neighborhood structure of fuzzy point and Moore-Smith convergence, Zike Deng in 1982, studied the fuzzy point and discussed the fuzzy metric spaces with the metric defined between two fuzzy points, [4].

George and Veermamani [5], Kramosil and Michalek have introduced the concept of M-fuzzy topological spaces induced by M-fuzzy metric spaces which have very important applications in quantum practical physics particularly in connections which were given and studied by El-Naschie

The main objective of this paper is to study and prove the completeness of fuzzy metric spaces using M-fuzzy metric spaces.

1- Basic concepts and preliminaries:

we start with the following definition of D-metric spaces , which plays an important role in this paper.

Definition (1.1), [6]:

Let X be a non empty set. A generalized metric (or D-metric) on X is a function $D : X\times X\times X \longrightarrow \square^{+}$, that satisfies the following conditions for each x, y, z, a $\in$ X:

(1) $D(x, y, z) \geq 0$.

(2) $D(x, y, z) = 0$ if and only if $x = y = z$.

(3) $D(x, y, z) = D(p\{x, y, z\})$, where p is the permutation function.

(4) $D(x, y, z) \leq D(x, y, a) + D(a, z, z)$.

The pair (X, D) is called the generalized metric or D-metric space.

Definition (1.2), [7]:

A D-metric space (X, D) is said to be D-bounded if there exists a positive real number N, such that $D(x, y, z) \leq N$, for all x, y, z $\in$ X.

In such a case N is said to be the D-bound for X. Moreover, if $E \subseteq X$, then E is said to be D-bounded subspace of X if there exists a positive real number M, such that $D(x, y, z) \leq M$, for all x, y, z $\in$ E.

Now, an illustrative examples are considered for completeness purpose.

Example (1.3), [1], [6]:

Let $X = \square$ and $D(x, y, z) = |x - y| + |y - z| + |z - x|$, for all x, y, z $\in$ X. Then (X, D) is unbounded D-metric space.

Since if we let x, y, z, a $\in$ X, then:

i- $D(x, y, z) = |x - y| + |y - z| + |z - x| > 0$ if and only if two of x, y, z are distinct and if $x = y = z$ then $|x - y| + |y - z| + |z - x| = 0$ and hence $D(x, y, z) = 0$

if $D(x, y, z) = 0$, then $|x - y| + |y - z| + |z - x| = 0$, which is true iff $|x - y| = 0$, $|y - z| = 0$, $|z - x| = 0$ and therefore $x = y = z$.

ii- $D(x, y, z) = |x - y| + |y - z| + |z - x|$

$= |x - z| + |z - y| + |y - x|$

$= D(x, z, y)$

Similarly, $D(x, y, z) = D(x, z, y) = D(z, x, y) = \ldots$

i.e., $D(x, y, z) = D(p\{x, y, z\})$, where p is the permutation function.

iii- $D(x, y, z) = |x - y| + |y - z| + |z - x|$

$\leq |x - y| + |y - a| + |a - x| + |a - z| + |z - z| + |z - a|$

$= D(x, y, a) + D(a, z, z)$

Therefore (X, D) is a D-metric space.

But if there is no positive real number N, such that $D(x, y, z) \leq N$, for all x, y, z $\in$ X, then (X, D) is unbounded D-metric space.

Lemma (1.4),[8]:

Let (X, D) be a D-metric space, then $D(x, x, y) = D(x, y, y)$.

The next definition introduces the fuzzy metric spaces by using M-distance functions

Definition (1.5), [6]:

A 4-tuple $(X, M_D, *)$ is called M-fuzzy metric space if X is an arbitrary (nonempty) set, * is M-continuous T-norm and M is a fuzzy subset of $X\times X\times X\times(0, \infty)$, satisfying the following conditions for each $x, y, z, a \in X$ and $t, s > 0$:

1. $M_D(x, y, z, t) > 0$.
2. $M_D(x, y, z, t) = 1$ if and only if $x = y = z$.
3. $M_D(x, y, z, t) = M_D(p\{x, y, z\}, t)$, where p is a permutation function of x, y and z.
4. $M_D(x, y, a, t)*M_D(a, z, z, s) \leq M_D(x, y, z, t + s)$.
5. $M_D(x, y, z, *) : (0, \infty) \longrightarrow [0, 1]$ is a continuous.

Lemma (1.6),[8]:

Let (X, d) be a metric space and consider the M-fuzzy metric space $(X, M_d, *)$ and define M_d by:

$$M_d(x, y, t) = \frac{t}{t + d(x, y)}$$

Then $M_d(x, y, t + s) \geq M_d(x, y, t)$, $\forall$ s, t, > 0.

The next lemma shows that condition (4) of definition (1.5) may be proved in all cases and thus this condition may be violated from the definition.

Lemma (1.7),[8]:

Let $(X, M_D, *)$ be an M-fuzzy metric space. Define $M_D : X\times X\times X\times(0, \infty) \longrightarrow [0, 1]$, by:

$$M_D(x, y, z, t) = M_d(x, y, t)*M_d(y, z, t)*M_d(z, x, t)$$

Then:

$$M_D(x, y, z, t + s) \geq M_D(x, y, a, t)*M_D(a, z, z, s)$$

for every $t, s > 0$ and $x, y, z \in X$.

Proof:

Since:

$$M_D(x, y, z, t) = M_d(x, y, t)*M_d(y, z, t)*M_d(z, x, t)$$

Then:

$$M_D(x, y, a, t) = M_d(x, y, t)*M_d(y, a, t)*M_d(a, x, t) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1.1)$$

and

$$M_D(a, z, z, s) = M_d(a, z, s)*M_d(z, z, s)*M_d(z, a, s) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1.2)$$

and hence by definition (2.3.2):

$$M_D(x, y, z, t + s) = M_d(x, y, t + s)*M_d(y, z, t + s)*M_d(z, x, t + s)$$

$$\geq M_d(x,y,t)*M_d(y,a,t)*M_d(a,z,s)*M_d(z,a,s)*M_d(a,x,t)$$

$$= M_D(x, y, a, t)*M_d(a, z, s)*M_d(z, a, s) \text{ (using eq.(3.1))}$$

$$= M_D(x, y, a, t)*M_d(a, z, s)*M_d(z, a, s)*1$$

$$= M_D(x, y, a, t)*M_d(a, z, s)*1*M_d(z, a, s)$$

$$= M_D(x, y, a, t)*M_d(a, z, s)*M_d(z, z, s)*M_d(z, a, s)$$

$$= M_D(x, y, a, t)*M_D(a, z, z, s)$$

which follows from definition (1.6) and eq.(1.2). ■

Among the main results in this paper are the following two lemmas:

Lemma (1.8),[8]:

Let (X, D) be a D-Metric space, and let:

$$M_D(x, y, z, t) = \frac{t}{t + D(x, y, z)}, \; t > 0$$

where:

$$D(x, y, z) = |x - y| + |y - z| + |z - x|$$

Then $(X, M_D, *)$ is a fuzzy metric space.

Lemma (1.9),[8]:

Let $X = \square$ and let:

$$M_D(x, y, z, t) = \frac{t}{t + D(x, y, z)}, \; t > 0$$

where:

$$D(x, y, z) = \max\{|x - y|, |y - z|, |z - x|\}, \; \forall \; x, y, z \in X$$

Then $(X, M_D, *)$ is M-fuzzy metric space.

The next lemmas are given for completeness without proof ,since their proofs are given in their correspondence references further more, these results gives and details properties of M-fuzzy metric spaces

Lemma (1.10), [6]:

Let $(X, M_D, *)$ be an M-fuzzy metric space. Then $M_D(x, y, z, t)$ is nondecreasing with respect to t, for all $x, y, z \in X$, where:

$$M_D(x, y, z, t) = M_d(x, y, t) * M_d(y, z, t) * M_d(z, x, t)$$

Lemma (1.11),[9]:

Let $\{U_n, n \in W\}$, be a sequence of subsets of $X\times X\times X$, such that $U_0 = X\times X\times X$, where W is any index set and U_n contains the diagonal (the identity relation is called the diagonal), and $U_{n+1}oU_{n+1}oU_{n+1} \subset U_n$, $\forall$ n, where o denotes the composition of three uniformly M-continuous functions is a given

uniformly M-continuous. Then there is a non-negative real valued function d on $X\times X$, such that:

a. $d(x, z) \leq d(x, y) + d(y, z)$.

b. $U_n \subset \{(x, y) \mid d(x, y) < 2^{-n} = f(x, y)\} \subseteq U_{n-1}$, for each $n \in$ □ and if each U_n is symmetric (i.e., $U = U^{-1}$).

Then there is a pseudo-metric d satisfying condition (b).

Lemma (1.12), [9]:

If X is a uniform space which has a countable base, then X is pseudo-metrizable.

Proof:

If X has a uniformity U with countable base $\{U_n\}$, then by the principle of mathematical induction, we can construct a subsequence $\{U_n\}$, such that:

1. Each U_n is symmetric.
2. $U_noU_noU_n \subseteq U_{n-1}$.
3. $U_n \subseteq V_n$, $\forall$ n $\in$ □ .

Hence $\{U_n\}$ form a base for U and hence by the metrization lemma (1.11), we have a uniform space (X, U) is a pseudo-metrizable. ■

Lemma (1.13), [9]:

A regular T_1-space whose topology has a σ-locally finite base is metrizable

2-Main results

In this section, the statement and the proof of the completeness of M-fuzzy metric spaces are given which seems to be a normal results up to our know lodge. But first we need to start and prove some other results

Lemma (2.1), [10]:

Let $(X, M_D, *)$ be a M-fuzzy metric space. Then τ_M is a Hausdorff topological space and for each $x \in X$, $\{B(x, 1/n, 1/n) \mid n \in \square\}$ is a neighborhood base at x for the topology τ_M.

From the above lemma one can note that every fuzzy metric space indeed is a fuzzy Hausdorff space.

Theorem (2.2):

Let $(X, M_D, *)$ be an M-fuzzy metric space, and let:

$$M_D(x, y, z, t) = \frac{t}{t + D(x, y, z)}, \; t > 0$$

where:

$$D(x, y, z) = |x - y| + |y - z| + |z - x|$$

then (X, τ_M) is metrizable fuzzy topological space.

Proof:

For each $n \in \square$, define:

$$U_n = \{(x, y, z) \in X\times X\times X \mid M_D(x, y, z, 1/n) > 1 - \frac{1}{n}\}$$

where:

$$M_D(x, y, z, t) = \frac{t}{t + D(x, y, z)}$$

$$= \frac{\frac{1}{n}}{\frac{1}{n} + |x - y| + |y - z| + |z - x|}$$

It is sufficient to prove that the sequence $\{U_n\}$ is a base for a uniformity U on X, whose induced topology coincides with τ_M.

First, for each $n \in \square$, to prove that:

$$\{(x, x, x) \mid x \in X\} \subseteq U_n,\ U_{n+1} \subseteq U_n \text{ and } U_n = U_n^{-1}$$

Since:

$$M_D(x, x, x, t) = \frac{t}{t + |x - x| + |x - x| + |x - x|}$$

$$= \frac{t}{t} = 1$$

Hence $M_D \geq 1$ and therefore $\{(x, x, x) \mid x \in X\} \subseteq U_n$, i.e., the diagonal is contained in U_n.

Now, to prove that $U_{n+1} \subseteq U_n$, $\forall\ n \in \square$, and since n + 1 > n, hence $\frac{1}{n+1} < \frac{1}{n}$ and so:

$$1 - \frac{1}{n+1} > 1 - \frac{1}{n}$$

Therefore $U_{n+1} \subseteq U_n$ and $U_n = U_n^{-1}$

If $U = U^{-1}$, then U is called symmetric.

On the other hand, for each $n \in \square$, there is, by the M-continuity of *, $m \in \square$ such that m > 3n. Hence:

$$\frac{1}{m} < \frac{1}{3n}$$

and with * to be the usual product, gives:

$$\left[1-\frac{1}{m}\right]*\left[1-\frac{1}{m}\right]*\left[1-\frac{1}{m}\right] = \left[1-\frac{1}{m}\right]\left[1-\frac{1}{m}\right]\left[1-\frac{1}{m}\right]$$

$$< \left[1-\frac{1}{3n}\right]\left[1-\frac{1}{3n}\right]\left[1-\frac{1}{3n}\right]$$

$$< \left[1-\frac{1}{n}\right]\left[1-\frac{1}{n}\right]\left[1-\frac{1}{n}\right] < 1-\frac{1}{n}, \forall\, n \in \square$$

Therefore $U_m o U_m o U_m \subseteq U_n$ (by lemma (1.12))

Indeed, let $(x, y) \in U_m$, $(y, y) \in U_m$ and $(y, a) \in U_m$

Since $M_D(x, y, z, *)$ is non decreasing (by lemma (1.10))

Then $M_d(x, a, 1/n) \geq M_d(x, a, 3/m)$, and so:

$$M_d(x, a, 1/n) \geq M_d(x, y, 1/m) * M_d(y, y, 1/m) * M_d(y, a, 1/m)$$

$$\geq \frac{\frac{1}{m}}{\frac{1}{m}+|x-y|} * \frac{\frac{1}{m}}{\frac{1}{m}+|y-y|} * \frac{\frac{1}{m}}{\frac{1}{m}+|y-a|}$$

$$\geq \frac{\frac{1}{m}}{\frac{1}{m}+|x-y|} * \frac{\frac{1}{m}}{\frac{1}{m}+|y-a|}$$

$$\geq \left[1-\frac{1}{m}\right]*\left[1-\frac{1}{m}\right]$$

$$\geq 1-\frac{1}{n}$$

Therefore, $(x, a) \in U_n$ and thus $\{U_n : n \in \square\}$ is a base for a uniformity U on X.Since for each $x \in X$ and each $n \in \square$

$$U_n(x) = \{y \in X : M_D(x, y, y, 1/n) > 1-\frac{1}{n}\}$$

$$= B(x, 1/n, 1/n)$$

Hence from lemma (2.1), that the fuzzy topology induced by U coincide with τ_M.

By lemma (1.13), (X, τ_M) is a metrizable fuzzy topological space. ■

Definition (2.3), [11]:

An M-fuzzy metric space is said to be completely M-fuzzy metrizable if every M-fuzzy Cauchy sequence is M-fuzzy convergent.

Theorem (2.4), [10]:

Let $(X, M_D, *)$ be a M-complete fuzzy metric space, and let:

$$M_D(x, y, z, t) = \frac{t}{t+D(x,y,z)}, \ t > 0$$

where:

$$D(x, y, z) = |x-y|+|y-z|+|z-x|$$

Then (X, τ_M) is M-completely fuzzy metrizable.

Definition (2.5), [10]:

An M-fuzzy metric space $(X, M_D, *)$ is called precompact if for each r, with $0 < r < 1$ and each $t > 0$, there is a finite subset A of X, such that:

$$X = \bigcup_{a \in A} B(a, r, t)$$

In this case, M is called a precompact M-fuzzy metric space on X.

Definition (2.6),[3,12,13]:

A fuzzy topological space is compact if and only if each open cover of the space has a finite subcover.

Theorem (2.7):

Compact M-fuzzy metric space is M-complete.

Proof:

Suppose that $(X, M_D, *)$ is a compact fuzzy metric space, for each r, with $0 < r < 1$ and each $t > 0$ the open cover $\{B(x, r, t) : x \in X\}$ of X, has a finite subcover by definition (2.6)

Hence $(X, M_D, *)$ is precompact (by definition (2.5)

On the other hand, every M-Cauchy sequence $\{x_n\}$ in $(X, M_D, *)$ has a limit point $y \in X$

Let $\{x_n\}$ be a fuzzy M-Cauchy sequence in $(X, M_D, *)$ having a limit point $x \in X$, then there is a subsequence $\{x_{kn}\}$ of $\{x_n\}$ that M-converges to y with respect to τ_M.

Thus, given r, with $0 < r < 1$ and $t > 0$, there is $n_0 \in$ □ , such that for each $n \geq n_0$

$$M_D(x, x, x_{kn}, \frac{t}{3}) > 1 - s, \text{ where } s > 0$$

Which satisfies $(1 - s)*(1 - s) > 1 - r$

Also, there exists $n_1 \geq k(n_0)$, such that for each n, $m \geq n_1$

$$M_D(x_n, x_n, x_m, \frac{t}{3}) > 1 - s$$

Therefore, for each $n \geq n_1$

$$M_D(x, x, x_n, t) \geq M_D(x, x, x_{kn}, \frac{t}{3})*M_D(x_{kn}, x_n, x_n, \frac{t}{3})$$

$$\geq (1 - s)*(1 - s)$$

$$> 1 - r$$

Hence the fuzzy M-Cauchy sequence $\{x_n\}$ M-converges to x.

Thus $(X, M_D, *)$ is an M-complete fuzzy metric space. ■

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Effect of Purified 1-Hydroxyphenazine Pigment on B rosette formation against Secondary hydatidosis

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Abstract

The effect of purified 1-hydroxyphenazine pigment which was generated from *Pseudomonas aeruginosa* on specific immune response B cells inside the body of white BALB/C mice against experimental secondary hydatidosis and the infectivity of protoscoleces was studied.

In comparison with negative control mice groups (P.B.S.) the results showed that the higher purified concentrations (100) μmole/ml of this pigment had suppressive effect on this specific immune response B cells(B-Rosette formation) and this effect was highly significant after (6) weeks from challenge dose with protoscoleces intraperitoneally (I.P) against this pigment, and this effect reflects the protoscoleces infectivity which increased due to suppression of B rosette formation while the mitogen Phytohaemagglutinin (PHA) showed a significant stimulation of this specific humoral response which leads to decrement in protoscoleces infectivity in comparison with higher pigment concentrations .

KEYWORDS: 1-hydroxyphenazine.B cell. Rosette formation. Hydatid. Cyst.

تأثير ألصباغ ١- هيدروكسي فينازين النقيه في ألتشكل الزهري البائي ضد ألخمج التجريبي بالاكياس العداريه

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الملخص

تمت دراسة تأثير الصباغ ١-هيدروكسي فينازين ألمعزوله والنقيه من Pseudomonas aeruginosa في التشكل الزهري البائي داخل جسم الفئران البيض BALB/C ضد الخمج التجريبي بالاكياس العدارية ومدى تأثيرها على خمجية الروؤيسات البدائيه (infectivity of protoscolices) .

اظهرت النتائج مقارنة مع مجموعة السيطرة (P.B.S) بان التراكيز العاليه لهذه الصباغ (100) مايكرومول/مل لها تأثيرا مثبطا على ألتشكل الزهري للخلايا ألبائيه، وان هذا التأثير قد ازداد بصوره معنوية بعد مرور ستة اسابيع من ألخمج التجريبي بجرعة التحدي بالروؤيسات البدائية وان هذا التأثير يعكس مدى خمجية(Infectivity) هذه الرؤيسات والتي ازدادت لتثبيط فعالية التشكل ألزهري للخلايا أللمفاويه ألبائيه ، فيما أظهر ألمشطر أللانوعي (PHA) تحفيزآ في ألأستجابه ألمناعيه ألخلطيه ألمتخصصه والتي أدت الى نقصان في خمجية ألرؤيسات ألبدائيه مقارنة مع تراكيز الصباغ ألعاليه.

كلمات مفتاحيه-1-هيدروكسي فينازين. خلايا بائيه. تشكل زهري.عدري.كيس.

1- Introduction

Echinococcosis or hydatidosis is the most serious world wide human zoonotic disease caused by larval stage hydatid cyst of the dog tapeworm *Echinococcus granulosus* (1) ,which is widespread in Mediterranean region (2).

Despite inducing host cellular and humoral immune response this parasite is highly successful parasite that develops progress and ultimately causes chronic disease (3). This parasite secretes some antigens that are thought to be responsible for immunomodulatory activities promoting its survival within a mammalian host (4). These parasites have extraordinary abilities to control host immune rejection mechanisms and defending themselves from host human attack (5).

1-1.*Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic human pathogen of immunocompromised individuals. It is typically infects the pulmonary tract causing both pulmonary damage and high mortality rates in patients with cystic fibrosis and other forms of bronchiectasis (6).

Pseudomonas aeruginosa produces a number of virulence factors. The most common products secreted by this bacterium are phenazine pigment exotoxins such as pyocyanine and its metabolite 1-hydroxyphenazine (7), or secretes a variety of pigments, including, fluorescein (yellow-green and fluorescent, now also known as pyoverdin), and pyorubin (red-brown) (8).

Some studies had shown the ability of *P. aeruginosa* to produce phenazines is critical for killing parasites (9), fungi (10) and induction of neutrophil apoptosis (11).

In addition to the phenazine pigment, this pathogen generates other virulence factors that affect the immune system during infection causing both acute and chronic diseases, these factors are either enzymes like elastase (12), or maybe toxins like lipopolysaccharide (13), fluorescin (14) or mucoid substances like alginate (15).These products have biological effects on host cells that may contribute to some inflammatory states like apoptosis in respiratory epithelial cells (16), or immunological effects on some of the specific immune response cells and its products like T lymphocytes (17) or B lymphocytes (18), and interleukins (19), while others may affect some of the innate immune response elements like macrophages (20) and complement (21).

1-2. B lymphocytes.

In mammals B lymphocytes are specific immune cells that are developed primarily in the bone marrow and fetal liver. They mature there before proceedings via circulation to the

secondary lymphoid organ like lymph node, spleen and mucosal- associated lymphoid tissue (MALT) and in these secondary organs they start to produce circulating antibodies when it stimulated with either mitogens or T-independent antigenic stimulation (22).

These specific humoral immune cells during early maturation undergoes several immunoglobulin gene rearrangements that establish B-cell specific receptors before it travels to the secondary lymphoid organs and the blood in which interacts with antigen that triggers cell division and formation firstly plasma cell which produce large amount of specific antibody which bind to that specific antigen and secondly memory cell which responsible for anamnestic response (23).

One of these receptor is C3 receptor which enable B-cell to bind with erythrocyte coated with antibody and complement (EAC) forming rosette shape and this EAC rosette complex formation is due presence of receptor on B-cell for C3 and such rosette do not formed by T cells (24). This test is considered one of the antibody-dependant humoral measurements (25).

The aim of this study is to investigate the effect of this phenazine pigment (1-hydroxyphenazine) produced by P. aeruginosa on one of the specific immune cell- reaction against experimental hyadatidosis in vivo and the possible effect on the infectivity of the protoscolices.

2- Materials and Methods.

2.1. Source of protoscolices

All hydatid cysts were collected from patients resident in some of Baghdad hospitals (Iraq), and protoscoleces were isolated aseptically from cysts according to the method of (26). The number was adjusted to 2000 protoscoleces/1ml of sterile phosphate buffered saline (PBS; pH = 7.2) and their viabilities were determined according to the method adopted by (27) using eosin stain.

2-2. Design of experiments:

The inbred males (Females excluded) BALB/C mice groups were prepared to be injected as follow:

Four groups were inoculated intraperitoneally (I.P) with four purified concentrations of 1-hydroxyphenazine (25, 50, 75, 100) µmole/1ml (28). After seven days they were given the same concentrations as a booster dose of the pigment, and after same period they were infected (I.P) with 2000 protoscolices/1mL (P.B.S) as a challenge dose .The fifth group was inoculated (I.P) with 1mL of sterile (PBS) and used as negative control group. The sixth group was inoculated (I.P) with (100µgm/ml) non-specific mitogen Phytohaemagglutinin (PHA) and challenge dose with same

number of protoscolices and used as positive control. After (2, 4, 6) weeks B-lymphocytes were separated according to [29] method and mixed with Erythrocyte-Antibody-Complement (EAC) according to [30] method. Thin films of this mixture on very clean slides were done after (4 hour) incubation for B- rosette. All the films were fixed with 70% alcohol and stained with Wright-Giemsa stain. They were examined microscopically and 200 lymphocytes were counted. B-rosette forming cells (At least 3-5 SRBC bound to B Lymphocytes) were considered positive & counted. After 25 weeks all mice were killed and dissected under dissecting microscope and the infectivity of protoscoleces was investigated and recording cysts number and their diameters using vernier micrometer.

2-3. Statistical Analysis:

The suitable statistical methods were used in order to analyze and assess the results; they include the followings (31):

2-3-1 Descriptive statistics:

Summary statistic of the readings distribution (mean, SD, SEM, minimum & maximum).

2-3-2 – Inferential statistics:

These were used to accept or reject the statistical hypotheses, they include the followings:

Analysis of variation ANOVA (f-test).

Least significant difference LSD (f-test).

Note: The comparison of significant (P-value) in any test were: S= Significant difference (P<0.05).

HS= Highly Significant difference (P<0.01).NS= Non Significant difference (P>0.05).

2-3-3-Computer & programs:

All the statistical analysis was done by using Pentium-4 computer through the SPSS program (version-10) and Excel application.

3- RESULTS:

After two weeks of mice groups exposure to protoscoleces as a challenge dose, 1-hydroxyphenazine caused decrement in B- rosetting formation and this decrement was highly significant (P<0.01) specially among mice groups which exposed to high concentrations (100) µmole/ml of pigment which were (11.2±1.461) for B rosettings, while low concentration(50,25)µmole showed no significant difference (P>0.05) in B-rosetting formation in comparison with negative and positive control groups.(Table-1), and this decrement is continue highly significant(P<0.01) in B rosetting formation for mice groups which were exposed to (100)µmole/ml (9.50±1.472) after 4 weeks in comparison with negative control group PBS (Table-1).

Pigment concentration (75)µmole/ml showed high significant decrement P<0.01 in B- rosetting formation after 6 weeks of the challenge dose(11.25±3.653) in comparison with

negative control group PBS and the positive control groups PHA, while the mice groups exposed to (100))µmole/ml showed highly Significant decrement(P<0.01) in B rosettings formation(5.3±2.855)in comparison with both negative and positive control groups (Table-1).

The results reflect the infectivity of protoscoleces according to cyst growth and development (numbers and diameters) in comparison with PHA which show significantly decrease the infectivity of protoscoleces, but this decrement of infectivity was sometimes less or not significant(P>0.05) between some concentrations with respect to the cysts diameters (Table - 2).

Table-1-Effect of purified 1- hydroxyphenazine on B rosettings in *vivo* after 2, 4 and 6 weeks from protoscoleces infection.

Pigment concentrations µmole/ml	B- Rosettings After 2 weeks Mean ± S.D	After 4 weeks Mean ± S.D	After 6 weeks Mean ± S.D
P.B.S (- control)	22.4 ± 0.337	22.7±0.683	20.6±2.439
P.H.A(+ control)	26.2 ± 1.883	26.4±5.635	24.2 ±4.918
25	22.0 ±2.160 $	22.2±3.090 $	22.0 ±4.243 $
50	21.6 ±1.566 $	22.0 ±6.218 $	11.4 ±1,377 *
75	21.8 ±4.062 $	11.2 ±5.472 *	11.25 ±3.653 *
100	11.2 ±1.461 *	9.50±1.472 *	5.30 ±2.855 *

* HS= P<0.01 # S = P< 0.05 $ NS = P>0.05

Table- 2- Effect of purified 1- hydroxyphenazine pigment on cysts numbers and diameters after 25 weeks from protoscoleces infection.

Pigment concentrations µmole/ml	Cysts numbers		Cysts diameters(mm)	
	Mean ± S.D		Mean ± S.D	
P.H.A(+control)	1.66 ± 0.3633		1.838 ± 0.8222	
25	3.55 ± 0.4743	$	1.888 ± 0.6745	$
50	7.35 ± 1.9971	#	2.813 ± 1.2135	$
75	14.63 ± 7.3268	*	2.875 ± 5.4600	$
100	19.13 ± 0.8662	*	3.131 ± 0.9482	#

* HS= P<0.01 # S = P< 0.05 $ NS = P>0.05

One way ANOVA of cyst number showed P=0.00 highly significant (P<0.01.)

One way ANOVA of cyst diameter showed P=0.136 Non-Significant (P>0.05).

4-Discussion.

The ubiquitous host range of *Echinococcus* Metacestode exemplifies the extraordinary ability of these parasites to control host immune rejection mechanism (32).

From all above, the results showed that the higher concentrations of 1- hydroxyphenazine reduce rosettings phenomenon, while PHA is a good phytomitogenic which able to stimulates and proliferates T lymphocytes. These T cells secret cytokines in turns activated B lymphocytes (33). The protoscolices with PHA both are a good non-specifically mitogenic for unprimed T and B lymphocytes *in vitro.* (34).

No studies were found about the effect of this pigment(1-hydroxyphenazine) which isolated and purified from *Pseudomonas aeruginosa* on B lymphocytes rosetting formation as immunomodulators against parasites especially against secondary experimental hydatidosis but, generally, (35) found that the concentration (12.5)µmole/ml of phenazine derivative pyocyanine had suppressive effect on interleukin-2(IL_2) production, which play very important role in proliferation and differentiation of B-lymphocytes , and this effects increased proportionally with pigment concentrations.

These results agree with (28,36,37),which they said that all phenazine derivatives had suppressive effect on B-rosette formation and this effect depend on concentration that used in that experiment because the higher concentrations affect the CD16 which considered as B-cell surface receptor for EAC complex which in turns reduce the percentage of B-rosetting.

B-rosette formation is one of specific humoral immune responses which depend on B lymphocyte. These

cells have both FC-Receptor and surface immunoglobulins receptors (Sig), so, the increment of the antigen concentration and time of exposure may reduce the ability of these cells to bind with EAC complex to form B-rosette shape due to the saturation of (CD16) surface receptors of these cells which is considered as receptor for EAC complex [30].

This study agree with [18] who said that the higher concentrations of phenazine pigment has ability to suppress the B cell differentiation to antibody forming cells due to the suppression of (IL-2) receptor on B-Cell which is important in B cell proliferation and differentiation.

Finally, the mechanism of phenazine pigment was not well known [38] and till now numerous questions regarding this mechanism remain unanswered [39]. In summary, our results demonstrate that the *P. aeruginosa* pigment, 1-PH, induces suppression B rosetting phenomenon (especially at higher concentrations) against experimental hydatidosis in mice which is associated with a significant increase in the virulence of the protoscoleces in mice. *P. aeruginosa* may pave the way for the infection with the hydatid cysts. Alternatively, the existing hydatid infection may become more aggressive in patients colonized with some strains of this bacterium which secretes phenazine pigment.

Further studies are needed to understand the mechanism by which the pigment suppresses the immune response in vivo, and really many researches now carried on to see the effects of low concentrations of purified phenazine pigments which produced by this pathogen and may be modulates the immune response against experimental hydatidosis[40] .

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