Australian Journal of Basic and Applied Sciences, 11(13) October 2017, Pages: 148-157

AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com

Histological, Fluorescent &Electron microscope studies for evaluation carbon accumulation in lung of birds in polluted area in Wasit province

[1]Ahmed Al- badri and[2]Hadeel Al-Karadi

[1]Assistprofessor,Department of Biology, Faculty Science, Wasit University, Kut, Wasit, Iraq.
[2]Al-Karadi, Department of Biology, Faculty Science, Wasit University, Kut, Wasit, Iraq.

Address For Correspondence:
Ahmad Mahdi Al-badri,Assist professor,Department of Biology, Faculty Science, Wasit University, Kut, Wasit, Iraq.

ARTICLE INFO

Article history:
Received 10 August 2017
Accepted 1 October 2017
Available online 18 October 2017

Keywords:
Fluorescent microscope, Carbon, AO/EB stains, Lungs, Ducks, Scanning electron microscope.

ABSTRACT

BACKGROUND: The aim of this study is to detect anthracosis of lung and determination its appearance in different area of groups of ducks.Using histological dyes to detect deposits of carbon particles and acridine orange \ ethidium bromide (AO/EB) stains to express apoptosis as well as using scanning electron microscope to know the morphological shaped of carbon. **OBJECTIVE:** The accumulation of carbon in lung cause disease called anthracosis. Anthracosis is an old disease observed in mummies. It may be seen as a superficial black discoloration or scattered foci of black spots, which retract mucosa inward due to the effect of neighboring anthracotic lymphadenopathy. **RESULTS:** The histological examination on lungs samples that take from three different area of Wasit province shown the presence of carbon accumulated in alveoli macrophage in small amount in normal area while in brick factories area, the carbon nanotubes found in two types:Single-walled carbon nanotubes (SWCNT) and Multi-walled carbon nanotubes(MWCNT)whereas in Al-ahdab oil field only the first one is presence. On the other hand, scanning electron microscope show themorphological shape and types of carbon nanotubes (MWCNT and UFBC) that deposit in both polluted areas (Al-ahdab oil field and brick factories area). Fluorescence microscope analysis appeared the presence of early and late (progress)apoptosis in alveoli lung cells but in brick factories area more than apoptosis that found in Al-ahdab oil field. **CONCLUSION:** We believe that the amount of carbon that is thrown into the air from a brick factories more than the amount of carbon put forward byAl-ahdab oil field. This confirmedthrough histological result and grossly appearance during the dissection of the ducks. Also we conclude that carbon has an unhealthy effect on lung of ducks that lead tooccurrence of apoptosis (early and late).

INTRODUCTION

Birds change from mammals because of particular structures in their respiratory system (Al- badri and Al-Salman, 2016). The avian respiratory system consists of the nasal cavity, larynx, trachea, Syrinx, bronchi, lungs and multiple air sacs (Dyce *etal*., 2010). Main roles of respiratory system in birds are exchange gases inflows and outflows, requirements of flight, voice output and stability temperature bird body, air moved across breathing passage since the nasal cavity to the larynx then carry on by the trachea and go into syrinx in addition to bronchi (Al- badri and Al-Salman, 2016).The lungs of the duck as bright red-triangular or quadrilateral-shape, not divided into lobes, and has in the upper border several rows of grooves caused by embedded the vertebral ribs (McLelland, 1990). In avian the lungs is specialized organ and differ from other organs by extension during the mechanisms for ventilation (Demirkan*et al*., 2006). The lung was firmly attached to the ribs that leaves deep costal impressions (Incei*et al*., 2012). The lung in ducks have three surfaces (Costal, Vertebral, and Septal) (Rastogi, 2007). The lungs is appears have parabronchi are opened into the several dilated chambers called atria and epithelium of air capillaries and the endothelium of blood capillaries were separated by basement membrane

ToCite ThisArticle:1Ahmed Al- badri and 2Hadeel Al-Karadi., Histological, Fluorescent &Electron microscope studies for evaluation carbon accumulation in lung of birds in polluted area in Wasit province. ***Aust. J. Basic & Appl. Sci.,*** *11(13): 148-157, 2017*

only (AL-Mussawy, 2011).The tertiary bronchus (parabronchi) and its surrounding tissues is considered as the respiratory portion of the lung.Mucosa of parabronchi lining Simple cuboidal or squamous epithelium and lamina propria Thin layer of fine connective tissue and the muscular layer consists of 3-5 layers of smooth muscle cells (El-Bab 2004).Carbon nanotubes (CNTs) hold great promise to create new and better products, but their adverse health effect is a major worry. Human exposure to Carbon nanotubes is primarily through inhalation and dermal contact, especially during the handling processes and manufacturing (Luanpitpong*et al*., 2014). There are three types of carbon nanotubes: Single-walled carbon nanotubes (SWCNT), Multi-walled CNT (MWCNT) and ultrafine carbon black (UFCB) (Mishra *et al*., 2015). Single-walled carbon nanotubes (SWCNT) Consisting of a rolled-up cylindrical sheet of graphene,Nanotubes can have multiple walls (MWNTs) - cylinders inside the other cylinders, and ultrafine carbon black (UFCB), spherical shape particles of (UFCB), are carbonaceous nanoparticles sharing fibrous morphology with a well-known, naturally occurring, toxic fiber, asbestos(Shvedova*et al*., 2013). The potential health risks of CNTs exposure have been elevated, related to the following reasons: their high aspect ratio and mode of exposure similar to asbestos fibers, inducing a concern about their potential fiber-like toxicity; their smallestnano-sized structure that makes them more reactive and toxic than larger particles; and their graphitic structure that is probable to have high durability and bio-persistence (Donaldson *et al*., 2006; Aschberger*et al*., 2010).The purpose of this study to know the relative proportion of carbon in oil field and brick factories areas and also know the apoptosis Which occurred as a result of the carbonaccumulated.

MATERIALS AND METHODS

Animals And Tissue Preparation:

Eighteen domestic ducks were collected from three different places in Wasit province. The birds are divided into three groups according to the area that collected from (Al-dejeli, Al-ahdab oil field and brick factories area) in Wasit . Each group contained six birds, all birds should be clinically healthy and devoid from any type of injuries. All domestic ducks were slaughtered, after that the lung samples were isolated completely from the thoracic region of the domestic ducks. All samples were used for light and electron microscope techniques. For histological study, several samples of the lungs were fixed in formalin 10% for 72 hours and washed up in tap water for 2-3 hours and then moved the samples to many histological techniques: dehydration, clearing, infiltration, embedding, cutting and staining with hematoxylin and eosin (H&E) stain for appearing the general structure of the tissue, in addition with periodic acid Schiff stain detect deposits of carbon particles in paraffin sections.

Fluorescent Microscope Method:

Using in this method Acridin Orange (AO) / Ethidium bromide (EB) stains to detect the apoptotic body. When this stain bound to DNA, the AO give the green color to cells, but the EB give the orange fluorescence. Acridine orange dye detects the live cells that suffered from the fragment (early apoptosis) whereas ethidium bromide is dye the cells that lose its cytoplasmic membrane (dead cells).The blocks are sectioning 6μm thickness by microtome and then remove paraffin wax from sections by put in xylene (10-15) minutes. Sections were rehydrated in ethanol (99%, 90%, and 70%) then were passed to distilled water. Added acridine orange / ethidium bromide to slides for 10 minutes. Rinse slides with phosphate buffer saline to remove stain & dry for a few minutes. Mounting with DPX mounting media.

Scanning Electron Microscope Technique:

For rigorous observation of carbon accumulation in the lower parts of respiratory system, ultrastructural study by scanning electron microscope was used for this purpose. The basic steps that used in Scanning electron microscope sample preparation involve surface cleaning, stabilizing the sample with a fixative, rinsing, dehydrating, drying, mounting the specimen on a metal holder, and coating the sample with a layer of a material that is electrically conductive. This test occurred in Iran, Tehran, Tehran University, Al-Razi laboratory.

RESULTS AND DISCUSSION

1. Histological results of lungs:

a- Al-dejeli area (normal area):

The mean of body weight of ducks (*Anas platyrhynchos*) was calculated. The mean of ducks in this area was about 1592 ± 110.43.

Table1: Shows the mean body weight (gm.) in three groups of domestic ducks.

The place of Ducks	*Body Weight (Mean ± SE)*
Al-dejeli area	1592 ± 110.43
Al-ahdab oil field area	1459 ± 141.83
Brick factories area	1652 ± 66.07

SE= standard error, n=6 and ($P \leq 0.05$) between different areas

The histological findings was appeared the lung have parabronchi were opened into the several enlarged chambers called atria and the endothelium of air capillaries and blood capillaries were separated by basement membrane only. The tertiary bronchus (parabronchi) and its surrounding tissues are considered as the respiratory portion of the lung. Mucosa of parabronchi lining Simple cuboidal or squamous epithelium and lamina propria Thin layer of fine connective tissue (Fig.1).All these results were agreement with some authors(Onuk*et al.*, 2009); (AL-Mussawy, 2011).

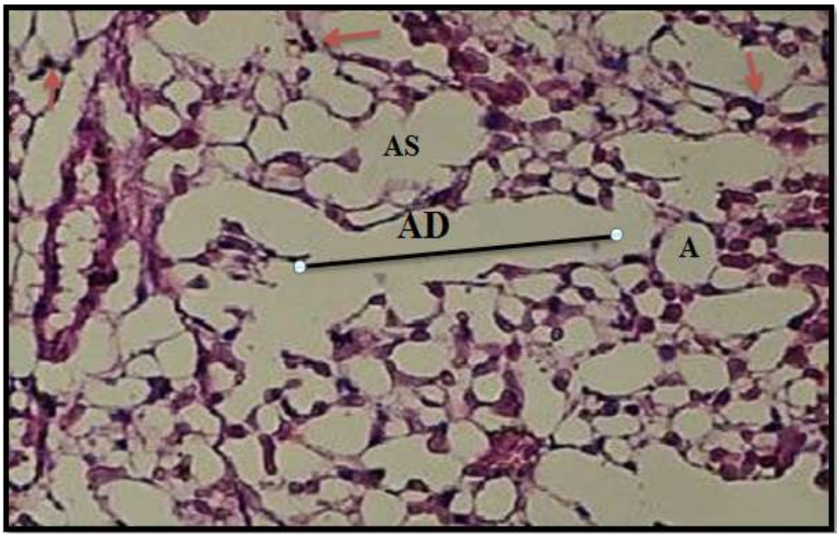

Fig. 1: The cross section of lung in Duck (in normal area) showing Alveoli (A), Alveoli duct (AD), Alveoli Sac (AS) and the arrow was showing carbon particles (H&E stain, 100X).

With H&E and periodic acid- Schiff (PAS) stain shown in this area, there was the little amount of macrophage contain carbon particles in alveoli (Fig.2, 3). The ratio of carbon aggregation in the lung of birds collected from normal area relatively was (+) (Table 2).

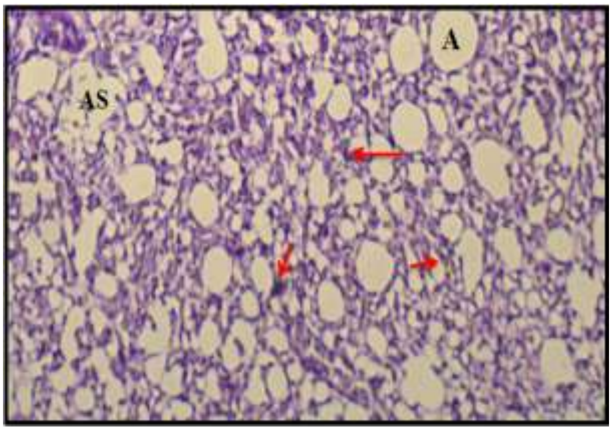

Fig 2: The cross section of lung in Duck (in normal area) showing Alveoli (A), Alveoli Sac (AS) and the arrow was showing the carbon particles (PAS stain, 40X).

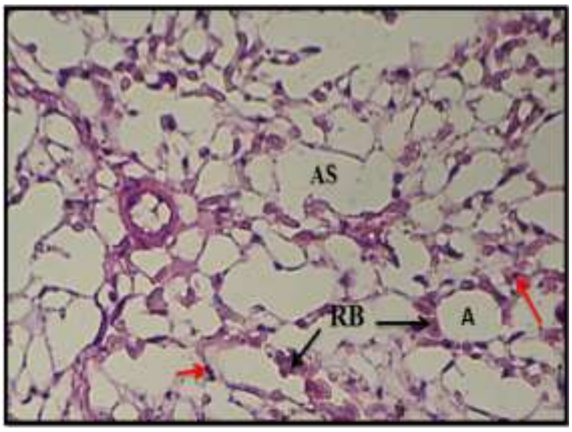

Fig. 3: The cross section of lung in Duck (in normal area) showing Alveoli (A), Alveoli Sac (AS), Nucleated Red Blood cells (RB) and the arrow was showing the carbon particles (H&E stain, 40X).

Thought this little proportion probably is due to the effects of consequents of agriculture machinery and vehicle which normally found in this area. Air pollution caused by vehicle exhausts, they observed that contaminants (soot) were considered to be major and produce through combustion of hydrocarbon fuel with both types gasoline and diesel.

b- Lung in brick factories and oil fields area:

Lung have a small cluster of alveoli (may be two or more) form blind pocketing known as alveoli Sac. There were two type of cells in alveoli, The first one known as type I pneumocyte or type I alveolar cell which was lined with squamous alveolar epithelial. The second one known as type II pneumocyte or type II alveolar cell which was different from type I lined with cuboidal alveolar epithelial, also pulmonary macrophage was present between alveoli cells (Fig.4). These statements were compactible with textbook of veterinary histology by (Samuelson, 2007); (Junqueira and Carneiro, 2005); (Ahasan*etal*., 2010)

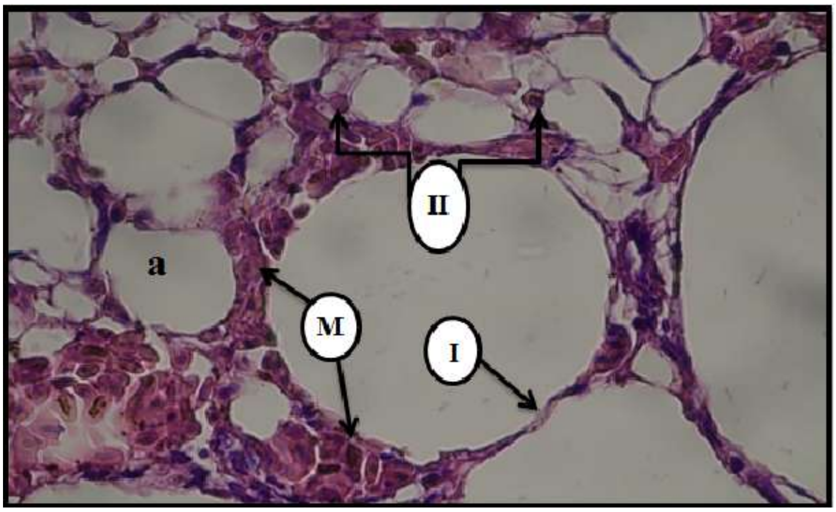

Fig. 4: The cross section of lung in Duck (in brick factories area) showing type I alveolar cell (I), type II alveolar cell (II) Macrophage (M) and alveoli (a) (H&E stain, 100X).

The histological and histochemical examination with different amount in the sections of lungs of birds which collected from areas polluted by remnants of brick factories and oil field (Fig. 5, 6).

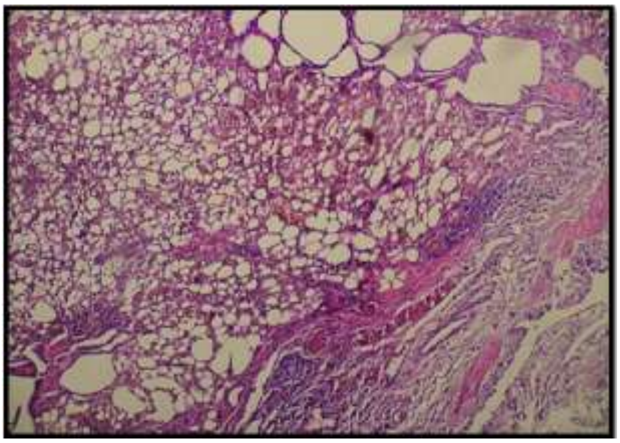

Fig. 5: The cross section of lung in Duck (in oil field) showing the aggregation of carbon (H&E stain, 20X).

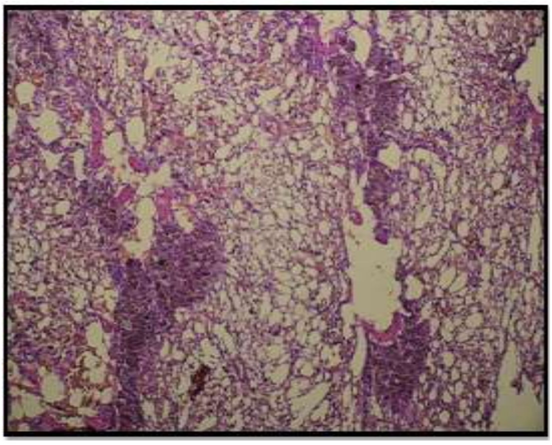

Fig. 6: The cross section of lung in Duck (in brick factories area) showing aggregation of carbon more than occurred in another area (H&E stain, 20X).

In the birds brick factories area, the lungs sections were contained two types of carbon nanotubes: single wall carbon nanotubes (SWCNT) and multi wall carbon nanotubes (MWCNT) (Fig. 7). Whereas only single wall carbon nanotubes was observed in the lung sections which collected from oil field areas (Fig. 8).

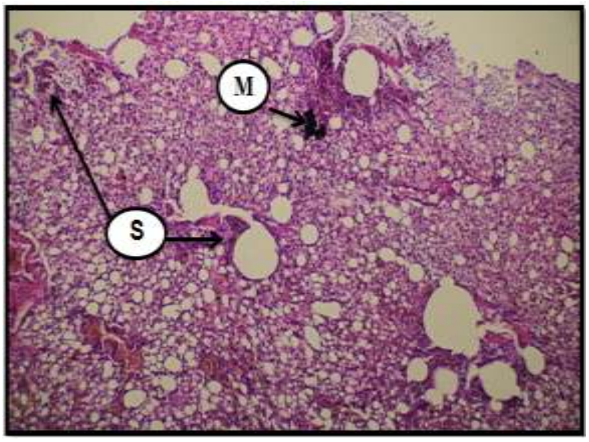

Fig. 7: The cross section of lung in Duck (in brick factories area) showing The Single wall carbon nanotube (S) and Multiwall carbon nanotube (M) (H&E stain, 20X).

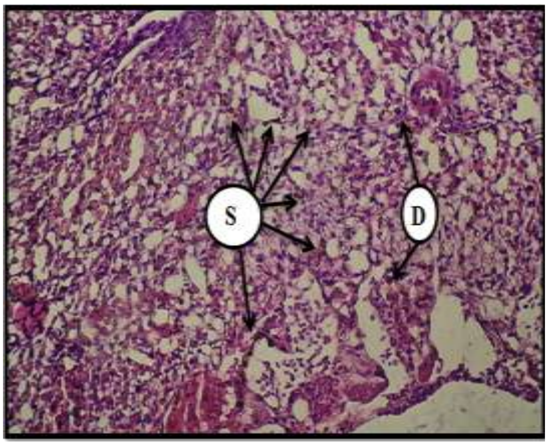

Fig. 8: The cross section of lung in Duck (in oil field) showing the Single wall carbon nanotube (S) and the Dust cells that engulf carbon(D) (H&E stain, 40X).

The histological analysis with (H&E) and PAS stains was showed that accumulations of carbon between cells of alveoli of lungs and its lymph nodes wereprofusely in brick factories samples compare with oil field samples (Fig. 9, 10, 11, 12, 13, 14), and relatively evaluated (+++) and (++) respectively (Table 2).

Table 2: Shows the relative proportion of carbon in trachea, bronchi and lung in three places examined birds (ducks).

Area of ducks	Lung
Normal area	+
Oil field area	++
Brick factories area	+++

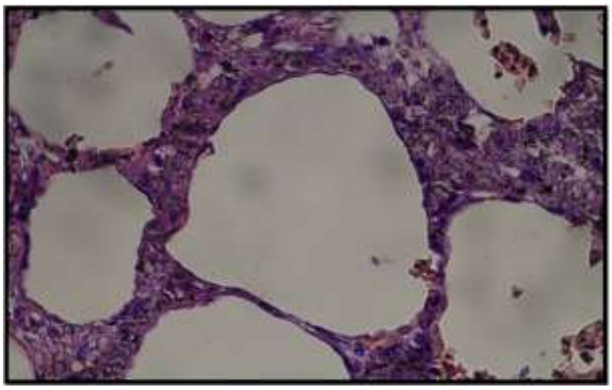

Fig. 9: The cross section of lung in Duck (in brick factories area) showing Carbon deposition in alveoli walls (H&E stain, 100X).

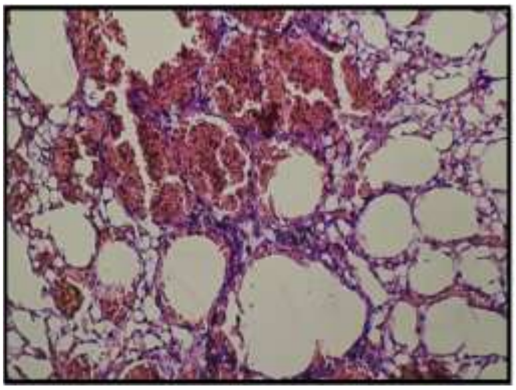

Fig 10: The cross section of lung in Duck (in brick factories area) showing Carbon deposition in alveoli walls (H&E stain, 40X).

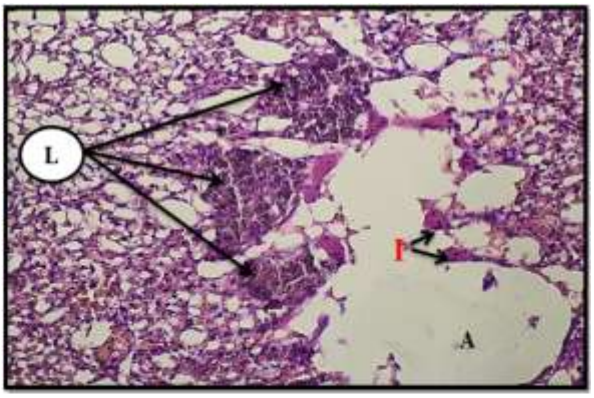

Fig. 11: The cross section of lung in Duck (in brick factories area) showing Carbon deposition in lymphocytes (L), Atrial opening(A) and interparabronchial septa (I) (H&E stain, 40X).

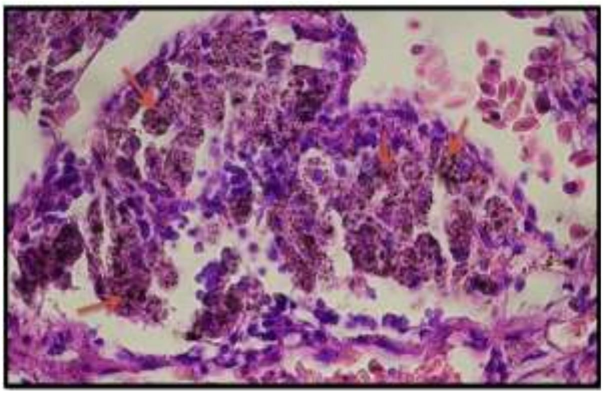

Fig 12: The cross section of lung in Duck (in brick factories area) showing Carbon was full all lymphocytes (H&E stain, 100X).

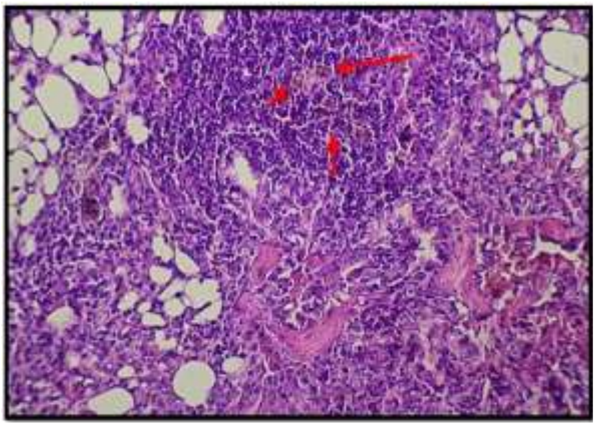

Fig. 13: The cross section of lung in Duck (in oil field area) showing Little carbon accumulation in lymphocytes (the red arrows) (H&E stain,

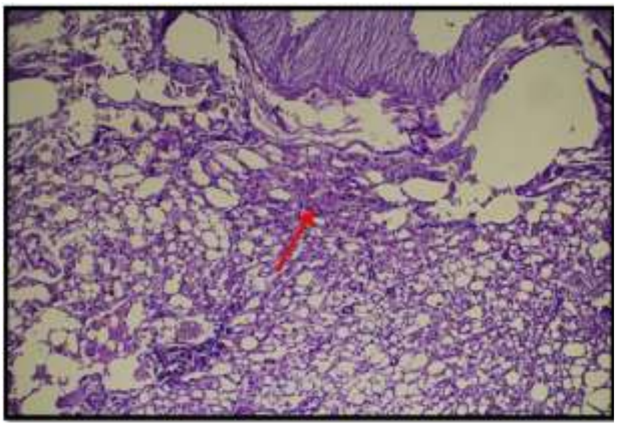

Fig. 14: The cross section of lung in Duck (in oil field area) showing very little carbon accumulation in lymphocytes (the red arrows) (PAS stain,

These findings were similar authors, (Choudary*et al*., 1986) they have been reported the anthracosis in different birds, reptiles and mammals kept with a catch in zoological park. While(Day*et al*., 1996) was described that the carbon aggregation in the lung (anthracosis) of dogs was rare.

(Ma-Hock*et al*., 2009)were proved through their experience there was black staining particles within macrophages, presumed to be MWCNT, located in the lymph nodes of the lung in rat. On the other hand, (Murray*et al*., 2012) they observed the presence of SWCNT in the lungs of mice. They believed that the SWCNT cause inflammation, pulmonary damage, oxidative stress in the lungs. (Mishra*et al*., 2015) They found both types of carbon nanotube SWCNT and MWCNT in the lungs of human also they explained that the both types were known to penetrate and persist in pulmonary interstitial tissue and directly contact with lung fibroblasts.

(Schoning*et al*., 1996); (Choudary*et al*.,1986) they said thatthe carbon aggregation in the lung tissue is considered to be more common in human than different animals because the farmers were more susceptible to be exposed to dust inhalation, such as coal mining and in factories discharging.

Theexamination of the fluorescent microscope usingAO / EB stain was confirmed the occurrence of early and late apoptosis in lung cells in both polluted areas high ratio of early apoptosis was appeared in the samples which collected from the oil fields area (++), whilst late stage of apoptosis was appeared in the samples of brick factories polluted area (++) (Fig. 15, 16) (Table 3).

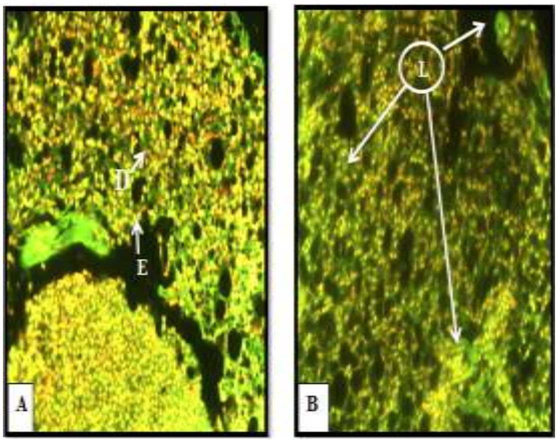

Fig. 15: The fluorescent microscope analysis of cross section of lung in Duck (in oil field area) showing A: late apoptotic cells (dead cells) (D) and early apoptotic cells

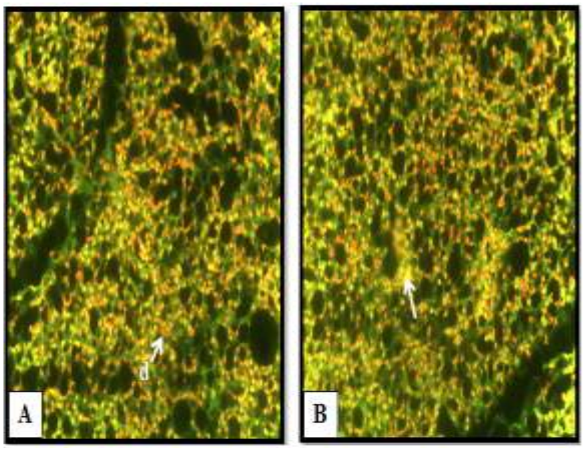

Fig. 16: The fluorescent microscope analysis of cross section of lung in Duck (in brick factories area) showing A: died cells (d) B: early apoptotic cells (arrow) (AO/EB stain, 40X).

Ravichandran*et al*., (2011) demonstrate through their study that the CNTs induce apoptosis in lung of mice due to activation of (apoptosis inducing factor) P53 and P21. We have shown that apoptosis occurred in the cellular structure of lung more than other respiratory air passage parts, the more suggestion that the last direction of the polluted air with foreign particles was the lungalveoli as well as the presence of alveoli macrophage.

Table 3: Shows the early and late apoptosis in the lung in polluted areas of examining birds (ducks).

Area of ducks	Lung	
	Early apoptotic	Late apoptotic
Oil field area	++	+
Brick factories area	+	++

2: Scanning Electron Microscope Results:

For observation of fine structure of the effected lungs were taken from brick factories and oil field areas, in addition to assess the morphology of carbon nanotubes (CNTs) the scanning electron microscope (SEM) were appeared two types of (CNTs), ball shape nanoparticles recognize the dispersed ultrafine carbon black (UFCB) and fiber shape morphology particles recognize multi wall carbon nanotubes (MWCNT) in the pulmonary tissue samples of polluted areas (Fig. 17, 18, 19,20).

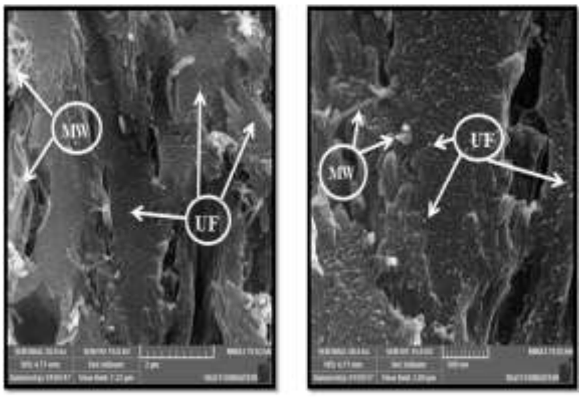

Fig. 17: Scanning electron microscope of lung in Ducks (in Brick factories area) showing Ultrafine carbon black (UF) and Multiwall carbon nanotube tube (MW).

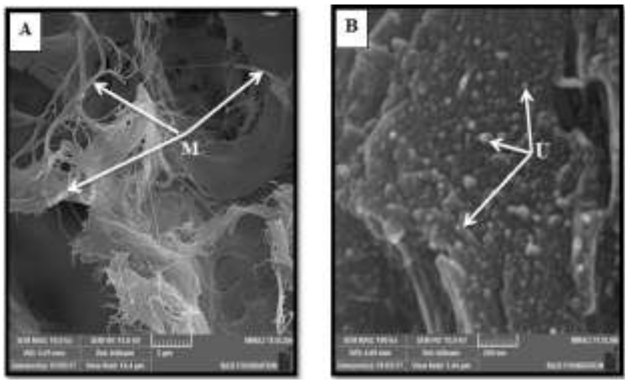

Fig. 18: Scanning electron microscope of lung in Ducks (in Brick factories area) showing A: Multiwall carbon nanotube tube (M). B: Ultrafine carbon black (U).

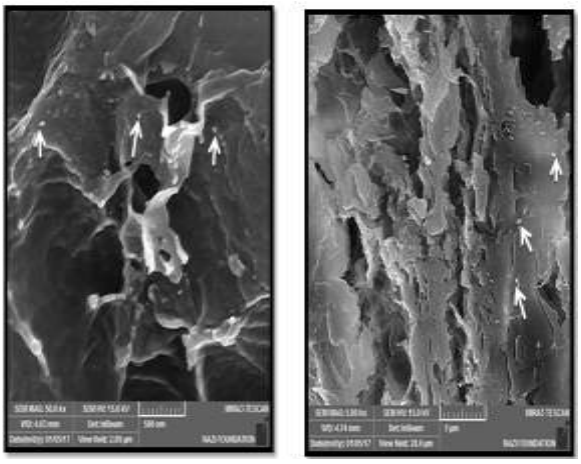

Fig. 19: Scanning electron microscope of lung in Ducks (in oil field area) showing Ultrafine carbon black (White arrow).

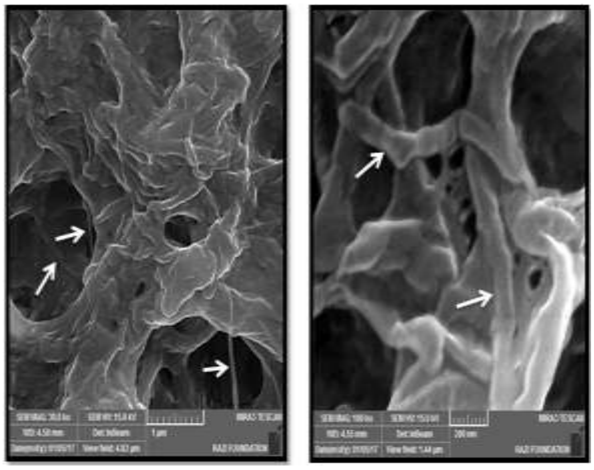

Fig. 20: Scanning electron microscope of lung in Ducks (in oil field area) showing Multiwall wall carbon nanotube (White arrow).

These results were similar to findings of Kasai*etal*., (2016) mentioned that the multi wall carbon nanotubes (MWCNT) was aggregation in pulmonary tissue and cause lung cancer in both male and female of rats. (Mishra*et al*.,2015) documented the (CNTs) appeared in three states, including (SWCNT),(MWCNT) and (UFCB), and mention that (MWCNT) had straight needle- shape morphology while spherical shape particles of (UFCB). (Købler*et al*.,2014) was described the large (CNT) and small (CNT) in the lung tissue of experimental mice.

The different shapes of carbon nanotubes particles which appeared in this results because these carbon particles composed of many nanotubes chemically bonded with each other, have electrically conductivity and possessing highly activated surface. All these shapes of CNT will necessary causes different abnormal cases and diseases of lungs. Whereas, (Mishra*et al*., 2015) reported the carbon nanotubes (SWCNT and MWCNT) induce fast interstial lung fibrosis. (Yang*et al*., 2010) mention that the multi wall carbon nanotubes passes through neuron cell membrane and neuron synthetic transmission. (Marchesan*et al*., (2015); (Tavares*et al*.,2015) they said that the toxicity of CNT can also effected according to the diameter of CNT based on single and multi-wall structure, while(Jia*et al*., 2005); (Visalli*et al*.,2015)mentioned that the (SWCNT) more toxically compare with (MWCNT) in macrophage, and inhaled (MWCNT) switch numerous toxicological pathway in epithelium of respiratory tract.

Conclusion:

We believe that the amount of carbon that is thrown into the air from a brick factories more than the amount of carbon put forward byAl-ahdab oil field. This confirmedthrough histological result and grossly appearance during the dissection of the ducks. Also we conclude that carbon has an unhealthy effect on lung of ducks that lead to occurrence of apoptosis (early and late).

The results of study of carbon aggregation in respiratory tract are summarize as flowing:- As a result of the presence of agricultural machinery and equipment beside vehicles in Al-dejeli area (normal area) we found little amount of carbon that precipitate in AM. The quantity of aggregated carbon in lung of brick factories area more than the amount of carbon deposition in lung section of oil field area. Single wall carbon nanotubes (SWCNT) and multi wall carbon nanotubes (MWCNT) were appeared in lungs of brick factories area, while in Al-ahdab oil field area only the single wall carbon nanotubes (SWCNT)was appeared in lung alveoli. Early and progress (late) apoptosis was appeared in lung cells in both areas (Al-ahdab oil field and brick factories areas) but the progress apoptosis in brick factories area was occurred more than in the Al-ahdab oil field. Scanning electron microscope explains the two types of carbon nanotubes: ultrafine carbon black (UFCB) and multi wall carbon nanotubes (MWCNT) were appeared in the lung of both areas but in different amount.

Recommendation:

Immunhisto-chemical study of carbon aggregation in respiratory tissues of birds. Study of carbon accumulation in the central nervous system (CNS) of animal which breeding in polluted area. Study of aggregation and effects of cadmium and Lead in the tissues of different systems for animals breeding in polluted area.

REFERENCES

Al- badri, A.M., A.A. Al-Salman, 2016. Histological and Immunohistochemistry Studies of Trachea Calcification in the Laying Hens (Gallus gallusdomesticus). International Journal of Scientific Engineering and Research, 4(7):11-16.

Ahasan, S.,E. Chowdhury,S. Azam, R. Parvin, A. Rahaman and A. Bhuyan, 2010. Pulmonary anthracosis in Dhaka Zoo collections-a public health forecasting for city dwellers. Journal of threatened Taxa, 2(11): 1303-1308.

AL-Mussawy, A.M.M., 2011. Anatomical and Histological Study of Major Respiratory Organs (Larynx, Trachea, Syrinx, Bronchi and Lungs) In Indigenous Male Turkey (Meleagrisgallopava). M.S. Thesis. AL-Qadisiya Uni. Vet. Med. College.

Aschberger, K., H.J.Johnston, V. Stone, R.J.Aitken, S.M.Hanken, S.A.K.Peters, C.L. Tran and F.M.Christensen, 2010. Review of carbon nanotubes toxicity and exposure—Appraisal of human health risk assessment based on open literature. Critical reviews in toxicology, 40(9): 759-790.

Choudary, C.H.,M.R.K. Mohan Rao and S. Ali, 1986. Anthracosis in zoo animals and birds. Indian Vet. J. 63: 869-870.

Day, M.J., G.R.Pearson, V.M.Lucke, S.J.Lane and R.S.J.Sparks, 1996. Lesions associated with mineral deposition in the lymph node and lung of the dog. Vet. Pathol., 33: 29-42.

Demirkan, A.Ç., R.M.Haziroglu and I. Kurtul, 2006. Air sacs (Saccipneumatici) in mallard ducks (Anas Platyrhynchos). Ankara Univ. Vet. Fak. Derg., 53: 75-78. 9.

Donaldson, K.,R. Aitken,L. Tran, V. Stone, R. Duffin, G. Forrest andA. Alexander, 2006. Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. ToxicologicalSciences, 92(1): 5-22.

Dyce, K.M., W.O.Sac andC.J.G.Wensing, 2010. Textbook of Veterinary Anatomy. 4th Edition. Saunders Elsevier. pp: 799-804.

El-Bab, M.R.F., 2004. Textbook Fundamentals of the Histology of Birds. 3ed edition. Assiut University,pp: 36-56.

Incei, N.G.,G. Pazvant and A. Hasan,2012. Anatomical features of the syrinx in sea gulls. Ankara Univ. Vet. Fak. Derg, p: 59.

Jia, G., H. Wang, L. Yan, X. Wang, R. Pei, T. Yan, Y. Zhao and X.Guo, 2005. Cytotoxicity of carbon nanomaterials: single - wall nanotube, multi-wall nanotube, and fullerene. Environmental science & technology, 39: 1378-1383.

Junqueira, L.C. and J. Carneiro, 2005. Basic histology text and atlas, London: McGraw Hill, Pp: 340-358.

Kasai, T.,Y,Umeda., M, Ohnishi., T, Mine., H, Kondo., T, Takeuchi., M, Matsumoto andS, Fukushima, 2016. Lung carcinogenicity of inhaled multi-walled carbon nanotube in rats. Particle and fibre toxicology. 13(1): 53.

Købler, C.,A.T. Saber, N.R. Jacobsen,H.Wallin,U. Vogel, K. Qvortrup andK. Mølhave, 2014 FIB-SEM imaging of carbon nanotubes in mouse lung tissue. Analytical and bioanalytical chemistry, 406(16): 3863-3873.

Luanpitpong, S.,L. Wang andY.Rojanasakul, 2014. The effects of carbon nanotubes on lung and dermal cellular behaviors. Nanomedicine, 9(6): 895-912.

Ma-Hock, L.,S. Treumann, V. Strauss,S. Brill,F. Luizi, M. Mertler,K. Wiench,A. Gamer, B. Van Ravenzwaay and R. Landsiedel, 2009. Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. Toxicological Sciences, 112(2): 468-481.

Marchesan, S.,K. Kostarelos, A. Bianco andM. Prato, 2015. The winding road for carbon nanotubes in nanomedicine. Materials Today, 18: 12-19.

McLelland, J., 1990. Respiratory system. In: A Colour Atlas of Avian Anatomy. Wolfe Publishing Ltd. Eng.,pp: 95-119.

Mishra, A., T.A.Stueckle, R.R. Mercer, R. Derk, Y. Rojanasakul, V. Castranova and L. Wang1, 2015. Identification of TGF-β receptor-1 as a key regulator of carbon nanotube-induced fibrogenesis. American Journal of Physiology-Lung Cellular and Molecular Physiology, 309(8): L821-L833.

Murray, A.R.,E.R. Kisin, A.V.Tkach, N. Yanamala,R. Mercer, S.H. Young, B. Fadeel,V.E. Kagan and A.A.Shvedova, 2012. Factoring in agglomeration of carbon nanotubes and nanofibers for better prediction of their toxicity versus asbestos. Part FibreToxicol, 9:1-10.

Onuk, B., R.M.Hazirogluand, M. Kabak, 2009. Gross anatomy of the respiratory system in goose (Anseranserdomesticus): Bronchi and saccipneumatici. Ankara Univ. Vet. Fak. Derg., 56: 165-170.

Rastogi, S.C., 2007. Respiration. In: Essential of animal physiology. New Age Inter. (P) Ltd.,pp: 263-285.

Ravichandran, P.,S. Baluchamy, R. Gopikrishnan, S. Biradar, V. Ramesh, V. Goornavar, R. Thomas, B,Wilson, R. Jeffers, J. Hall andG.T. Ramesh, 2011. Pulmonary biocompatibility assessment of inhaled single-wall and multiwall carbon nanotubes in BALB/c mice. Journal of Biological Chemistry, 286(34): 29725-29733.

Samuelson, D., 2007.Textbook of Veterinary Respiratory system. Saunders, Elsevier,pp: 224-249.

Schoning, P.,J.L. Abraham andB.R. Burnett, 1996. Silicate and metal dust in lungs of Greyhounds. Am. J. Vet. Res., 57: 1006-1009.

Shvedova, A.A., N. Yanamala, E.R. Kisin, A.V. Tkach, A. Murray, R.A.Hubbs, M.M. Chirila, P. Keohavong, L.P.Sycheva, V.E. Kagan andV. Castranova, 2013. Long-Term Effects of Carbon Containing Engineered Nanomaterials and Asbestos in the Lung: One Year Post Exposure Comparisons. American Journal of Physiology-Lung Cellular and Molecular Physiology, 306: L170-L182.

Tavares, A.P.,C.G.Silva, G.Dražić, A.M.Silva, J.M.Loureiro and J.L.Faria, 2015. Laccase immobilization over multi-walled carbon nanotubes: Kinetic, thermodynamic and stability studies. Journal of colloid and interface science, 454: 52-60.

Visalli, G.,M.P. Bertuccio, D. Iannazzo, A. Piperno, A. Pistone, A. Di Pietro, 2015. Toxicological assessment of multi-walled carbon nanotubes on A549 human lung epithelial cells. Toxicology in Vitro, 29: 352-362.

Yang, Z.,Z.W. Liu, R.P. Allaker, P. Reip, J. Oxford, Z. Ahmed and G. Ren, 2010. A review of nanoparticle functionality and toxicity on the central nervous system. Nanotechnology, the Brain, and the Future, Springer, pp: 313-332.