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DETECTION OF HEAVY METALS IN PARASITIZE DOMESTIC ANIMALS AS ENVIROMENTAL BIO INDICATORS

M.Sc. THESIS
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ABBREVIATIONS AND SYMBOLS

Abbreviations	Classification
HF	**:**Hydate Fluid
GL	**:**Germinal Layer
HC	**:**Healthy Control
AE	**:** Alveolar Echinococcosis
CE	**:** Cystic Echinococcosis
IBLT	**:** Infected Bovine Liver Tissue
LBGL	**:** Liver Bovine Germinal Layer
LBHF	**:** Liver Bovine Hydate Fluid
LSGL	**:**Liver Sheep Germinal Layer
LIT	**:**Lıver Infected Tissue
ICP-OES	**:**Inductively Coupled Plasma-Optical Emiission Spectroscopy
MRI	**:**Magnetic Resonance Imagine
LDR	**:** Linear Dynamic Range
RF	**:** Radio Frequency
PMT	**:**Photo Multi-plier Tube
MIP	**:**Microwave Induced Plasma
CID	**:**Charge Injection Device
CCD	**:**Charge Coupled Device
SD	**:**Standard deviation
NS	**:**Non significant

Symbols	Description
As	**:**Arsnic
Al	**:**Aluminum
Cd	**:** Cadmium
Hg	**:** Merccury
Pb	**:** Lead
=	**:**Equals is the Same as
≠	**:** Is not equal to is defferenr from
>	**:**Is greater than is more than exceeds is above
≥	**:** Is greater than or equal to is at least is not less than
<	**:** Is less than is fewer than is below
≤	**:**Is less than or equal to is at most does not exceed is not greater than is no more than

ABSTRACT

DETECTION OF HEAVY METALS IN PARASITIZE DOMESTIC ANIMALS ENVIROMENT BIO INDICATORS

M.Sc. Thesis

SALEH, MAMOON QADER

The Graduate School of Science of Siirt University
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Echinococcosis is a parasitic disease caused by infection with tiny tapeworms of the genus Echinococcus. Heavy metals tend to bioaccumulate in living organisms, such as cow and sheep and their accumulation has been a major concern particularly in the tissues of these consumed domestic animals. Biological or Environmental monitoring of heavy metals is an interesting and important fast growing area that compose of utilizing a number of parasitic organisms as environmental bioindicators. The study aimed at assessing the possible undesirable effects of the excessive concentration of heavy metals in these consumed animals on the public health. In addition to clarifying the possible protective role of these parasites in protecting their hosts from serious danger through intake the heavy metals by these parasites rather than the tissues of their hosts particularly liver and lungs. A total of 116 liver and lung samples (89 of Liver and 27 of lungs) were collected from the slaughters of Erbil and Koya cities. A total of (84) parasites samples in two forms; Hydatid fluid (HF) and germinal layer (GL) were extracted and purified. Quantification of the heavy metals [Aluminum (Al), Arsenic (As), Cadmium (Cd), Mercury (Hg) and lead (Pb)] was carried out using inductively coupled plasma (ICP) technique. The results revealed different concentrations of heavy metals in different parasitized animals' organs (liver and lungs) with Echinococcus granulous parasite.

As related with Arsenic (As) metal, it was detected in high concertation in parasitized bovine liver and sheep lungs tissues, which were almost two times higher in the parasite (HF) than the infected liver tissues and almost three times higher than healthy uninfected animals, $P \leq 0.05$; and were one and half times higher in the parasite (GL) than healthy uninfected sheep animals, $P \leq 0.05$. while the slightly higher significant accumulation of mercury (Hg) was detected in these parasitized animals (GL), $P \leq 0.05$. Lead (Pb) was also detected in highly significant concentration in parasitized bovine lungs (Hydatid fluid) which was three times, and two times higher than the infected tissues and healthy uninfected control, $P \leq 0.05$. While, very low concentrations of Aluminum (Al) and cadmium (Cd) in the parasite, in opposite to infected animal tissues and healthy uninfected animals, by recording highly significant levels, $P \leq 0.001$.

Whereas in a comparison between parasitized bovine and sheep's liver and lungs, Aluminum (Al) concentration was higher 7 times in parasitized sheep lungs compared with parasitized bovine lungs, $P \leq 0.05$. While the same significant concentration was detected for both of cadmium (Cd) and Lead (Pb) in these animals. Mercury (Hg) was significantly higher in sheep liver than bovine liver, $P \leq 0.05$. The same levels of Cadmium (Cd) were detected in both parasitized bovine's liver and lungs, $P \leq 0.001$, and similar lead (Pb) level was detected in sheep's liver and lungs $P \leq 0.05$. In conclusion, these significant results indicate the possibility of the role of Echinococcus parasite as environmental bioindicators and monitor of some heavy metals.

Keywords: Echinococcus granulous, hydatid fluid, germinal layer, As, Hg, Al, Cd, Pb, Erbil, Koya and ICP-OES.

ÖZET

YÜKSEK LİSANS TEZİ

ÇEVRE BİYOİNDİKATÖR OLARAK PARAZİTLENMİŞ EVCİL HAYVANLARDA AĞIR METAL DÜZEYLERİNİN BELİRLENMESİ

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Ekinokkoz (Kist Hidatik), Echinococcus granulosus (E. granulosus) ve aynı gruptan diğer parazitlerin sebep olduğu, bir hastalıktır. Ağır metaller, inek ve koyun gibi evcil hayvanların organizmalarında biyoakümülasyon eğilimindedir, bu birikim etleri insanlar tarafından tüketilen evcil hayvanların dokularında risk oluşturabilecek bir düzeye kadar çıkabilir. Biyoindikatör olarak davranan bazı parazit organizmalar aracılığı ile ağır metallerinin ibiyolojik veya çevresel olarak izlenmesi, son döenmlerde önem kazanan ve hızla gelişen bir araşatırma alanı olmuştur. Bu çalışmanın temel amacı, etleri insanlar tafaından tüketilen evcil hayvanlarda ağır metallerin aşırı konsantrasyonunun halk sağlığı üzerinde olası istenmeyen etkilerini değerlendirilmesidir. Bununla birlikte bu parazitlerin konakladığı hayvanların akciğer ve karaciğerlerinde biriken ağır metalleri emerek konaklarını ağır metallerin olası tehlikelerine karşı koruma potansiyellerinide ortaya çıkarmaktır. Erbil ve Koya şehirlerinin değişik mezbahalarından toplam 116 karaciğer ve akciğer numuneleri alındı, (89 akciğer ve 27 karaciğer). Toplanılan akciğer ve karaciğer numunelerinden; Hidatik sıvı (HF) ve germinal tabaka (GL) olmak üzere İki farklı formda toplam 84 parazit örneği ekstre edildi ve saflaştırıldı. Elde edilen bütün numunelerin ağır metal [Alüminyum (Al), Arsenik (As), Kadmiyum (Cd), Cıva (Hg) ve kurşun (Pb)] düzeyleri ICP-OES cihazı kullanılarak yapılmıştır. Sonuçlar, farklı parazite hayvanların organlarında (karaciğer ve akciğerlerde) Echinococcus granulosus paraziti ile farklı ağır metal konsantrasyonları ortaya çıkardı. Arsenik (As) metal ile ilgili olarak, Sığır ve koyun doku ve organlarından alınanan örneklerdeki düzey, bu organlardan izole edilen parazit örneklerindeki düzeyden HF için 2 kat, GL için 1,5 kat ve sağlıklı dokulardan alınan örneklerdeki düzeylerden 3 kat daha yüksek olduğu gözlemlenmiştir $P \leq 0.05$.

Hg ve Pb metalleri ile ilgil olarak ise; Organ ve dokulardan izole edilen parazit (GL) örneklerindeki düzeylerin organ ve dokulardan alınan örneklerdeli düzeylerden belirgin bir şekilde yüksek olduğu tespit edilmiştir P≤0.05.

Al ve Cd metalleri ile ilgili olarak elde edilen sonuçlar göre, organ ve dokulardan izole edilen parazit örneklerindeki düzey, hem organ ve dokulardan elde edilen örmekerdeki düzeylerden ve sağlıklı deneklerden alınan örneklerdeki düzeylerden daha düşük olduğu tespit edilmiştir P≤0.001. Bozağı ve koyun karaciğerleri karşılaştırıldığında, koyun karaciğerlerinden izole edilen parazit örneklerindeki Al düzeyi bozağı karaciğerlerinden izole edilen parazit örneklerindeki düzeylerden 7 kat daha yüksek olduğu tesbit edilmiştir. P ≤ 0.05. Aynı etki bu hayvanlar daki Cd ve Pb düzeyleri içinde elde edilmiştir P ≤ 0.05. Parazitli sığırların karaciğerlerinde ve akciğerlerinde aynı düzeyde Cd tespit edildi, P <0.001 ve koyun karaciğeri ve akciğerlerinde P <0.05'de benzer kurşun (Pb) seviyesi tespit edildi. Sonuç olarak, bu anlamlı sonuçlar, çevresel biyolojik indikatör olarak Echinococcus parazitinin rolünü ve bazı ağır metallerin izlenebileceğini göstermektedir.

Anahtar kelimeler: Echinococcus granulosus, hidatik sıvı, germinal tabaka, As, Hg, Al, Cd, Pb, Erbil, Koya and ICP-OES.

1. INTRODUCTION

Echinococcosis is one of the parasitic diseases which causes infection by tiny tapeworms of the genus Echinococcus Echinococcosis which can be classified as either alveolar echinococcosis or cystic echinococcosis.

The hydatid disease also, it is another name of the Cystic echinococcosis (CE). The infection of such type of disease can be occurred by the larval stage of Echinococcus granulosus parasite. The long tapeworm with an approximated 2-7 millimeter found in the dogs (definitive host) and sheep, goats, cattle, and pigs (intermediate hosts). The humans mostly infected by the CE which is very harmful by enlarging cysts in the liver, lungs, and other organs that often grow unnoticed and neglected for years.

The infection of the larval stage of Echinococcus multilocularis can cause the Alveolar echinococcosis (AE) disease which was found the approximately 1-4 millimeter long tapeworm in the coyotes, foxes, and dogs (definitive hosts). Also, it has intermediate hosts for E. Multilocularis in from of small rodents. The AE disease is very rare in the human but relatively common in the animals in endemic areas. The AE disease threatens human health than the CE disease by forming parasitic tumors in the lungs, liver, brain, and other organs in the human body and in the case of any neglection and untreated of AE disease the result will be a fatal. (Casaravilla, C. Malgor, R. and Carmona, C. 2005).

The most common brain parasitic infection is a brain echinococcosis which occurred in the rare location, in all cases can be represented from 1% to 2% with hydatid diseases.

The pediatric population and young adults with a male predominance infection commonly happened with such disease in the different percentage.

The hosts of Echinococcus are various carnivores and the man is an accidental host. The hydatid cysts are usually grown slow with the asymptomatic, and clinical manifestations of the hydatid cyst disease can be diagnosed by the C.T scan which caused by compression of the involving organs. The hydatid cyst can be seen in the spherical form, thin walled, homogeneous and non-enhancing cystic lesion without peripheral edema. Dowling technique is one of the most common technique to design the procedure for giving birth to the intact cyst by irrigating saline between cyst wall-brain interfaces. İn the case of with

preoperative rupture the medical treatment can be indicated in the more than one location. The Prognosis often is good, but the similar complications will occur after surgery. The Prognosis depends on the location, a number of cysts, size, and technique used.

The heavy metals are most toxic and harmful metals among the other elements, which are naturally occurring elements with the different concentrations in all Ecosystems. There are a large number of heavy metals exist in nature. They can be found in the elemental form and in the different chemical compounds.

The volatile substances become linked to very fine particles which can be widely imparted on the larger scale. The different properties of each form and compound of the heavy metals strongly affect the human by entering human food chains. The biochemical cycles and balance of some heavy metals drastically changed by human activates.

The production of lead, zinc, and copper increased tenfold from 1850 to 1990. (Nriagu, J.O.1988: CACAR 2003).

The pioneer heavy metals of worry to the Cooperative Programmer for controls and detection of the Long-range Transmission of Air Pollutants in Europe (EMEP) in the field of heavy metal pollution assessment are Hg, Pb and Cd, because they are the most poisonous and, have known serious effects on e.g. Human health. The Ecological exposure to high concentrations of heavy metals has been attached with various cancers and kidney damage. İn the Europe more considerable measurement data available on Hg, Pb, and Cd heavy metals. There are thirty-five metals that concern us because of their professional and residential exposure and twenty-three of them are heavy elements such as cerium, antimony, bismuth, arsenic, cadmium, chromium, copper, cobalt, gallium, iron, gold, lead, silver, tellurium, thallium, manganese, mercury, nickel, platinum, vanadium, uranium, and tin (Jaishankar, M., Tseten, T.,and Beeregowda, K.N., 2014).

The proper quantity of these heavy elements is very needful for maintaining good health, but at the same time the excess quantity of them causes chronic toxicity and poisoning. The excesses and lacking of heavy metals in the human body reason to damage lungs, kidneys, liver, central nervous system and damaging other vital organs.

In the case of long-term exposure by heavy elements can cause to tardily progressing physical, muscular, and neurological degenerative processes that mimic, Parkinson's disease, Alzheimer's disease, muscular dystrophy, and multiple sclerosis. Allergies are not uncommon and reiterate long-term exposure to some of heavy metals or their compounds may be cause to cancer (Takala, J. 1999) {International Occupational Safety and Health Information Centre 1999}. The toxic level of some of heavy metals can be just on top of the background concentrations naturally found in nature. Therefore, it is important to maintain human health providing safety guidelines against the heavy metals. The toxicity of heavy metals is the unfamiliar medical condition in the most of the accessories in the United States. however, it has the significant clinical condition when it occurs. The inappropriate treatments and unrecognized the poisoning by toxic heavy elements can cause to reduced fineness of life and significant illness (Ferner D.J., 2001) for those has been poisoned by toxic heavy metals. Therefore, an appropriate and suitable medical procedures should be taken (Dupler, D. 2001).

The symptoms of poisoning by acute toxicity can be recognized by several symptoms such as rapid in onset and severe pain associated to known exposition (Ferner D.J.2001) which causes of nausea, cramping, pain, vomiting, headaches, sweating, difficulty breathing, motor, weaken cognitive, and language skills, mania, and convulsions. The chronic exposure to heavy elements causes to appear which can be recognized. The chronic exposition symptoms are very analogous to symptoms of other health conditions and often cab is developed tardily over months or years. Also, it is very important to mention that the symptoms of chronic exposure to toxic heavy metals can lead the victim to neglect the symptoms because of relating them to other types of illness. (Ferner D.J., 2001).

2. LITERATURE REVIEW

2.1 The History of Echinococcosis

The history of echinococcosis in Europe includes a period of over 2000 years. Already in antiquity metacestodes (hydatids) of Echinococcus granulosus, the causative agents of Cystic Echinococcosis (CE), were observed in animals and humans. Alveolar Echinococcosis (AE), caused by metacestodes of E. multilocular is, was identified as a disease entity only in the middle of the 19th century. It took about 100 years until it was undoubtedly clarified and accepted that CE and AE are not caused by a single Echinococcus species, but by E. granulous and E. multilocular is, respectively.

In the 20th century, significant progress has been achieved in echinococcosis research, including diagnosis, epidemiology, therapy, immunology, molecular biology and other fields. However, CE and AE remain actual problems as in many endemic regions resources and structures are lacking for effective surveillance and control of these zoonosis threatening humans. Echinococcosis is a disease that has been recognized by humans for centuries. There has been mention of it in the Talmud. It was also recognized by ancient scholars such as Hippocrates, Aretaeus, Galen, and Rhazes.

Although echinococcosis has been well known for the past two thousand years, it wasn't until the past couple of hundred years that real progress was made in determining and describing its parasitic origin. The first step towards figuring out the cause of echinococcosis occurred during the 17th century when Francesco Reid illustrated that the hydatid cysts of echinococcosis were of "animal" origin. Then, in 1766, Pierre Simon Pallas predicted that these hydatid cysts found in infected humans were actually larval stages of tapeworms. (Torgersen, P.R., and Budke, C.M. 2003).

A few decades afterward, in 1782, Goeze accurately described the cysts and the tapeworm heads, while in 1786, E. granulous was accurately described by Bartsch. Half a century later, during the 1850s, Karl von Siebold showed through a series of experiments that Echinococcus cysts do cause adult tapeworms in dogs. Shortly after this, in 1863, E. multilocular is was identified by Rudolf Leuckart.

Then, during the early to mid-1900s, the more distinct features of E. granulous and E. multilocular is, their life cycles and how they cause disease were more fully described as more and more people began researching and performing experiments and studies. While E. granulous and E. multilocular is were both linked to human echinococcosis before or shortly after the 20th century, it wasn't until the mid-1900s that E. oligarchs and E. Vogel were identified as well and shown as being causes of human echinococcosis.(Moro, P.L., Nakao, M. and Cabrera, L., 2009).

2.1.1 Morphology

It's commonly known as (The dog tapeworm) or (The hydatid worm). Adult E. granulous was described by Hartmann in the small intestine of dog in1695. (Belding, D.L. 1965).

The adult worms reside in the small intestine of the dog and other carnivores (wolf, fox, and jackal), and are 3 to 6mm long. It consists of a scolex, neck, and strobila (3 to 4 proglottids). The dog and sheep are optimum definitive and intermediate hosts respectively.

Scolex: it is pyriform in shape and measures about 300mm in diameter. It possesses four suckers and a protrusible rostellum with two circular rows of hooklets. Neck: it is short and thick.

Strobila:it consists of three segments (occasionally four). The first segment is immature, the second is mature, and the third (and the fourth when present) is gravid.

The eggs measure at 32-36 μm in length and 25-32μm in width, and contains an embryo that is called an oncosphere or hexacanth (because it has three pairs of hooklets).

The Larval forms are funds within the hydatid cyst which develop in the intermediate host. (Karaeva RR, 2003) The body plan of adult Echinococcus In strobila several organs like the excretory system extend through the entire worm Proglottid: an individual segment.(Rahman, W.A. 2015)

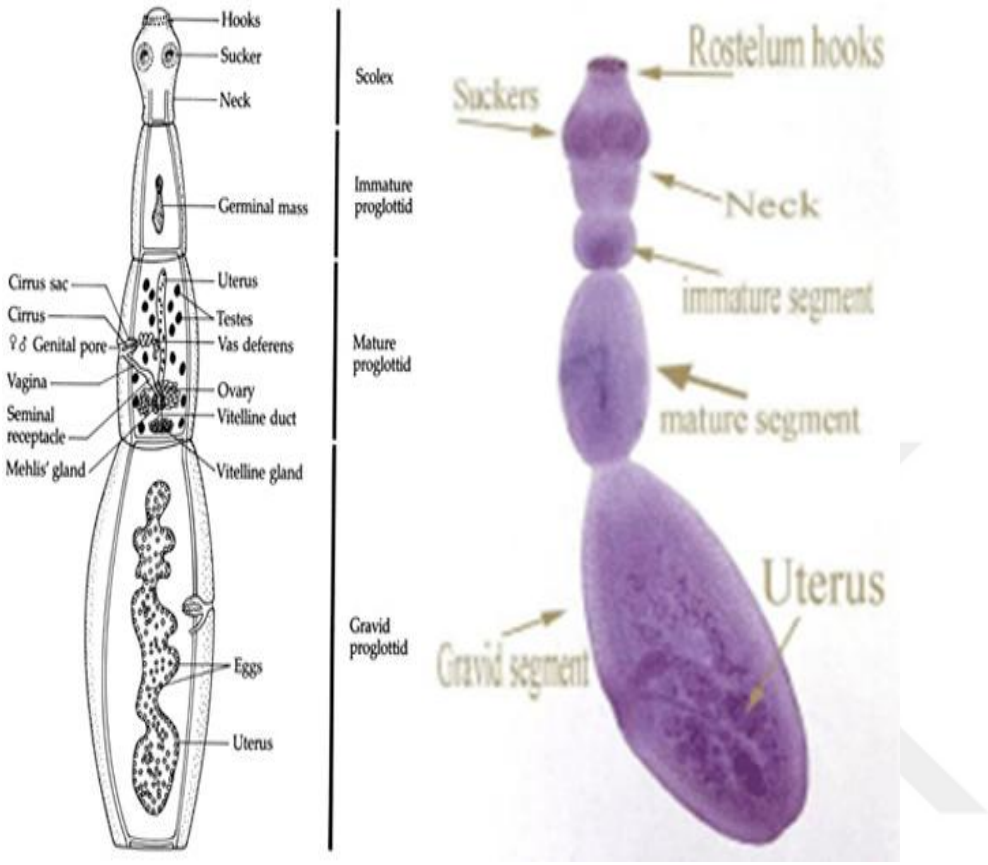

Figure 2.1.1. Morphology of adult worm of E. granulosus.

2.1.2 Life cycle

The adult Echinococcus granulose (3 to 6 mm long) resides in the small bowel of the definitive hosts, dogs or other canids. Gravid proglottids release eggs that are passed in the feces. After ingestion by a suitable intermediate host (under natural conditions: sheep, goat, swine, cattle, horses, camel), the egg hatches in the small bowel and releases an oncosphere that penetrates the intestinal wall and migrates through the circulatory system into various organs, especially the liver and lungs. In these organs, the oncosphere develops into a cyst that enlarges gradually, producing protoscolices and daughter cysts that fill the cyst interior.

The definitive host becomes infected by ingesting the cyst-containing organs of the infected intermediate host. After ingestion, the protoscolices evaginate, attach to the intestinal mucosa, and develop into adult stages in 32 to 80 days. (Thompson RA, 2002).

The same life cycle occurs with E. multilocular is (1,2 to 3,7 mm), with the following differences: the definitive hosts are foxes, and to a lesser extent dogs, cats, coyotes and wolves; the intermediate host are small rodents; and larval growth (in the liver) remains indefinitely in the proliferative stage, resulting in invasion of the surrounding tissues. With E.Vogeli (up to 5,6 mm long), the definitive hosts are bush dogs and dogs; the intermediate hosts are rodents; and the larval stage (in the liver, lungs and other organs) develops both externally and internally, resulting in multiple vesicles. E. oligarchies (up to 2,9 mm long) has a life cycle that involves wild felids as definitive hosts and rodents as intermediate hosts. Humans become infected by ingesting eggs, with the resulting release of oncosphere in the intestine and the development of cysts in various organs. (Sarkar, M., Pathania, R. and Chopra, R., 2016).

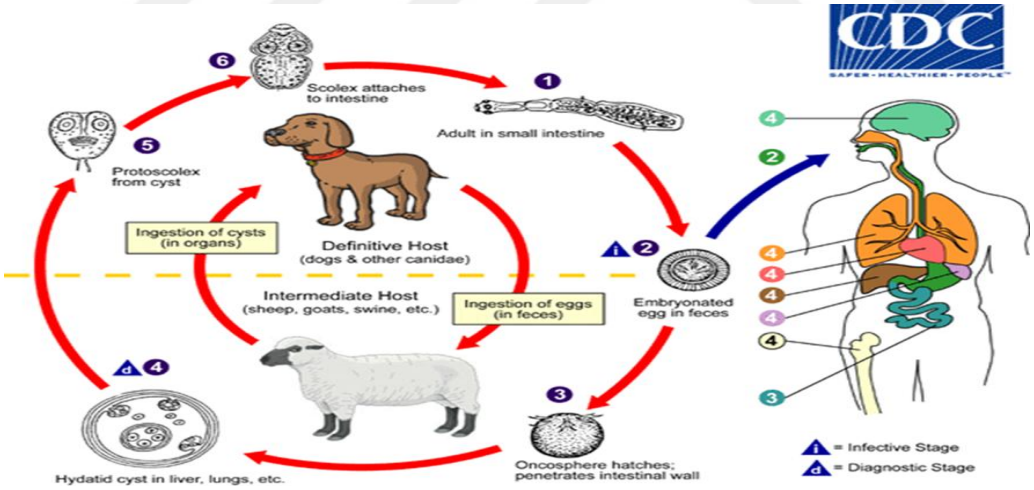

Figure 2.1.2. Illustration showing the life cycle of E.granulosus in both animals and humans.

The life cycle of the parasite in (animals) involves six stages:

1- The bowel of the dogs attached by adult worms resides.

2- The egg releases by gravid segments which are throughout in the dog faces.

3- The Ruminant animals will ingest eggs and releasing oncosphere by the hatch in their bowels, which invade the intestinal wall and travel through the circulating system to various organs of the host.

4-. The developer of oncosphere into Hydatid cysts in the site can produce Protoscolices and daughter cysts.

5- The Dogs can ingest the ruminant infected organs.

6-The intestinal wall of the dogs attached by protoscolices and begin to grow slowly into adults in during 32 to 80 days.

The life cycle of the parasite in (human) involves four stages:

1) The life cycle of the parasite in humans.

2) The contaminated eating food with Parasite eggs can infect human.

3) The oncosphere will be released by ingesting parasite eggs in the small intestine.

4) The migration of oncosphere carried out through the circulating system to various sites where they develop and produce hydatid cysts.

2.1.3.Pathogenicity

Persons with cystic hydatidosis often remain asymptomatic until hydatid cyst containing the larval parasites grow large enough to cause discomfort, pain, nausea, and puking. The vesicles grow over the course of several age before reaching adulthood and the rate at which symptom appear typically depends on the location of the cyst. The cysts are mainly uncovering in the liver and lungs but can also appear in the quick temper, kidney, heart, ivory, and central nervous arrangement, caused by psychic harm and may cause mild to a severe anaphylactic chemical reaction, even death, as an issue of the release of cystic fluid.

Alveolar consonant echinococcosis (AE) is characterized by parasitic tumors in the liver and may bed cover to other organs including the lung and nous. (Thompson RA. 1995).

In humans, the larval forms of E. multilocular are do not fully mature into cysts but cause vesicles that invade and destroy surrounding tissue and

Cause discomfort or pain, weight unit deprivation, and malaise. AE can cause liver unsuccessful person and death because of the spread into nearby tissues and, rarely, the brain. AE is a dangerous disease answer in a mortality rate of 50% and 75%, especially because most affected people live in remote control locations and have poor health care.

Persons with cystic echinococcosis often remain asymptomatic until hydatid cysts containing the larval parasites grow large enough to cause discomfort, pain, nausea, and vomiting. The cysts grow over the course of several years before reaching maturity and the rate at which symptoms appear typically depends on the location of the cyst.

The cysts are mainly found in the liver and lungs but can also appear in the spleen, kidney, heart, bone, and central nervous organization, including the brain and eyes. Cyst rupture is most frequently caused by trauma and may cause mild to severe anaphylactic reactions, even death, as a result, the release of cystic fluid. Alveolar echinococcosis (AE) is characterized. (Dziri, C., Haouet, K. and Fingerhut, A., 2004).

2.1.4 Prevalence and transmission

Hydatid disease is due to the larval form of Taenia hot domestic dog. The definite hosts of Echinococcus are various carnivore , the common being the dog, which develops the adult worm in the gut following ingestion of the larvae that are present in the tissues of the intermediate host (typically sheep and Capricorn and occasionally, mankind) and then go on to develop in the visceral tissue, particularly in the liver and lung . The man is an accidental host. He gets infected through the face-oral path by ingestion of food contaminated by dog feces containing ova of the parasite or by direct physical contact with Frank.

The embryos pass through the parties of the gut into the portal system and are carried to the liver where most larvae get entrapped and encysted. Some may reach the lungs and occasionally, some may pass through the capillary filter of the liver and lungs and get entry into the systemic circulation, to the brain. (Markos, A., 2013).

The cerebral hydatid vesicle is slow growing and present late when they gain in size and become large.

There is no consensus on the increase rate of the hydatid cyst of the brain and has been variably reported between 1.5 and 10 cm/year. (Brunetti, E., Garcia, H.H. and

Junghanss, T., 2011) Intracranial hydatid cyst may also be the classified advertisement as primary or secondary winding.

The primary cyst is formed as a solution to direct infestation of the larvae in the brain without a demonstrable involvement of other organs. In the human host, a hydatid cyst can lead to life history -threatening ramification, such as cyst rupture, with the spread of new cysts, and bacterial infection. There are some risk factors. Rural origin of adult patients was reported in over 70% of cases. (Strohmaier, W.L Wilbert, D.M. and Seitz, H.M., 1990). Exposure may be the result of contact with dogs from breeders, farmers, butchers, veterinary surgeon, or during recreational activities (hunting). Comorbidity is observed in some adult's patients such as hypertension, Echinococcosis is a zoonotic disease. Definitive hosts are carnivores such as dogs, wolves, and foxes. (Craig, P.S., McManus, D.P., 2007).

The adult worms mature in the small intestine of definitive hosts and shed proglottids in their feces. Upon ingestion of these eggs by intermediate hosts (herbivores such as sheep, horses, cattle, pigs, goats, camel, and humans), the oncosphere larva is released in the small intestine and penetrates the mucosa to enter the lamina propria. From there, passive transport occurs to target organs by hematogenous or lymphatic route, disseminating primarily to the liver, secondarily to the lung, and finally to other organs where it forms the hydatid cyst. The triple layered cyst consists of an inner germinal layer that gives rise to protoscolices, a middle cellular laminated layer and an outer host-tissue derived fibrous layer. As life cycle depends on carnivores eating infected intermediate hosts, humans are usually a dead end for the parasite.-In Libya, most human cases of CE are caused by sheep strain G1, and cattle strain G5 and camel strain G6. These intermediate hosts are the most common reared animals in the country. Dogs are an essential part of hydatid disease transmission to humans and other ruminant animals; however, vaccination of dogs provides a very practical and cost-effective Prevention strategy. (Bozdag, Z., Oguz, A, Gumus, M. 2015).

2.1.5 Diagnosis

Ultrasonography imaging is one of the technique can be used for the diagnosis of both alveolar echinococcosis and cystic echinococcosis. This technique is usually complemented or validated by computed tomography (CT) Scan and/or magnetic resonance imaging (MRI) scans Cysts can be incidentally discovered by radiography.

Specific antibodies are detected by different serological tests and can support diagnosis. Biopsies and ultrasound-guided punctures may also be performed for differential diagnosis of cysts from tumors and abscesses.

The infected dogs by echinococcosis can be performed diagnosis by separating the parasite from their small intestine after necropsy or their faces due to recent developments in immunodiagnostic assays the dogs infected by echinococcosis have been described. Therefore, to protect the public health and minimize the risk factors as well as treatment of hydatid disease the appropriate control strategies should take in consideration such as using the veterinary vaccines. (Casaravilla, C., Malgor, R., and Carmona, C., 2005).

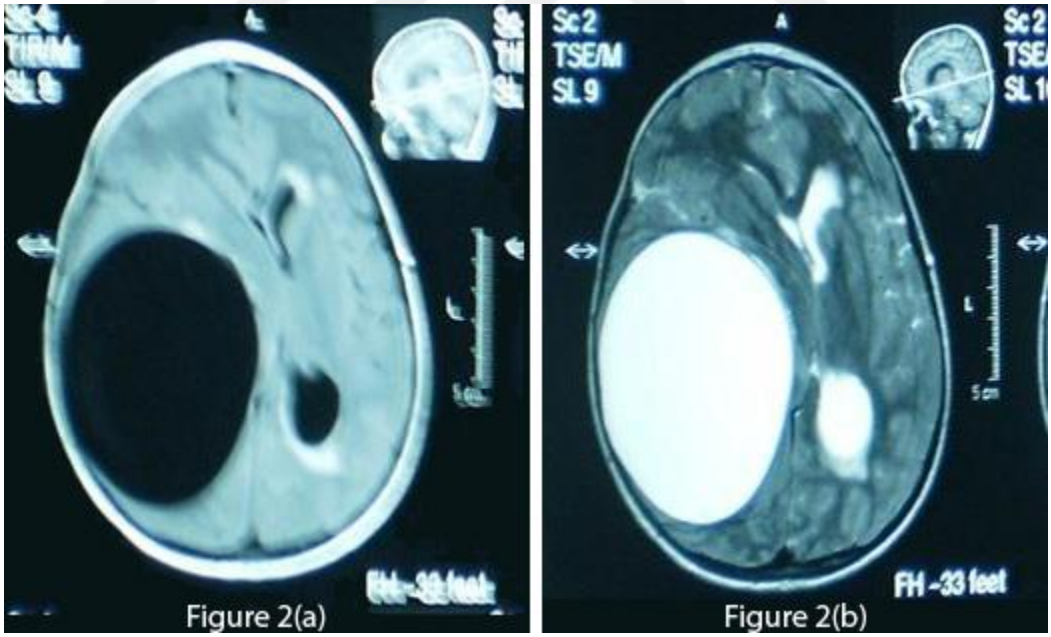

Figure 2.1.3. T1-weighted axial MRI of the brain demonstrates a cyst density similar to CSF.

Fig 2.1.4. (b): T2-weighted MRI shows no ring enhancement or oedema. The Periventricular hyper Intensity of the left side is probably due to Obstructive hydrocephalus.

In the intermediate host, diagnosis depends on the detection of the larval cyst form, which can occur in almost any organ, but particularly in the liver and lungs the diagnostic repertoire includes imaging techniques, mainly ultrasound (US) and computed tomography (CT) examination for abdominal echinococcosis and X-ray for lung echinococcosis, and immunodiagnostic tests. (Pawlowski, Z.S., Eckert, J. and Grimm, F., 2001).

The diagnosis of echinococcosis in dogs or other carnivores requires the demonstration of the adult custody of Echinococcus app. in their faces or the small intestine or the detection of specific copro antigens or copro-DNA. (Kassa, S.A., 2012).

2.1.6 Therapy

In the past, the Cystic Echinococcosis surgery was the only treatment for cystic Echinococcus cysts. The cyst puncture, chemotherapy, and PAIR (percutaneous aspiration, injection of chemicals and respiration) have been used to replace surgery as effective treatments for cystic echinococcosis and in some cases, there was no treatment but a conservative "watch and wait" approach is best. The treatment indications depend on the cyst characteristics such as cyst type, size, location, and complications.

The surgery probably is the best treatment for liver cysts which are secondarily infected or the cysts located in the lungs, brain, or kidney. The liver cysts larger than 7.5 cm are likely to have biliary communication can be eliminated by surgery. The injection of protoscolicidal chemical solutions uses of treatments of many abdominal cysts which followed by evacuation, prior to further manipulations and extirpation of cysts.

Today, treatment options for CE include surgery, PAIR (puncture, aspiration, injection, respirations) and chemotherapy. (Pawlowski, Z.S., Eckert, J. and Grimm, F., 2001).

Percutaneous drainage has been increasingly used as an alternative to surgery in the treatment of hydatid cysts. Surgical procedures ordinarily involve inactivation of the cyst contents, then the removal of all cyst components. (Yorganci, K. and Sayek, I., 2002).

Percutaneous drainage has many advantages; however, hydatid cyst membranes, which are composed of a laminar layer and a germ native layer, cannot be removed by this method. Percutaneous drainage may be performed by puncture, aspiration of cyst contents, injection of suicidal agents, and respiration of fluid, as described by Ben Amor et al (1986), or by catheterization, as described by Akhan and Özmen (1999). Regardless of which method is used, 5–10 years of follow-up is advocated because of the potential for recurrence or infection. (Akhan, O. and Özmen, M.N., 1999).

For some patients, chemotherapy with benzimidazoles is the preferred treatment Patients with small cysts or multiple cysts in several organs can be treated successfully with albendazole. Approximately one-third of patients treated with chemotherapy with Benzimidazole drugs have been cured of the disease and even higher proportions, between 30-50%, have responded with significant regression of the cyst size and alleviation of symptoms. Both albendazole 10 to 15 mg/kg body weight per day (max 800 mg orally in two doses) in several 1-month courses with treatment-free intervals of 14 days and, as a second choice for treatment, mebendazole 40-50 mg/kg body weight per day continuously have been Highly effective. Additionally, chemotherapy can be very effective when used in conjunction with surgery. (Lacey, E., 1990).

Albendazole has been administered to patients prior to surgery for the intended purpose of facilitating the safe surgical manipulation of the cysts by inactivating protoscolices, altering the integrity of the cysts membranes, and reducing the turgidity of the cysts. A third treatment option, PAIR (percutaneous aspiration, injection of chemicals and respiration), has been shown to be effective. This option is indicated for patients with relapse after surgery, failure of chemotherapy alone, or who refuse surgery.

Alveolar echinococcosis requires chemotherapy with or without surgery; radical surgery is the preferred approach in suitable cases. Effective treatment involves benzimidazoles administered continuously for at least 2 years and patient monitoring for 10 years or more since recurrence is possible. This has inhibited progression of alveolar echinococcosis and reduced lesion size in approximately half of treated cases. Intermittent treatment with albendazole is not recommended.

The most commonly done procedure designed to give birth to the intact cyst by irrigating saline between cyst wall-brain interfaces (Figure 1). This technique, reported by Dowling and improved by Arana-Iniguez, is possible because of minimal adhesions around the cyst wall. (Rexiati, M., Mutalifu, A., Azhati, B. and Wang, Y., 2014.).

Aspiration of the cystic contents through puncturing during the surgery for deep-seated cyst or cysts which located in critical areas is an alternative method, especially as multiple hydatid cysts resulting from the rupture of a primary cyst are infertile and have no broad capsule which permits the use of Dowling technique. Solitude product use, as hypertonic saline serum, is essential to protect the brain parenchyma and the risk of desalinization in cases of intraoperative rupture. For multiple and bilateral hydatid cysts the surgery must be done in several time.

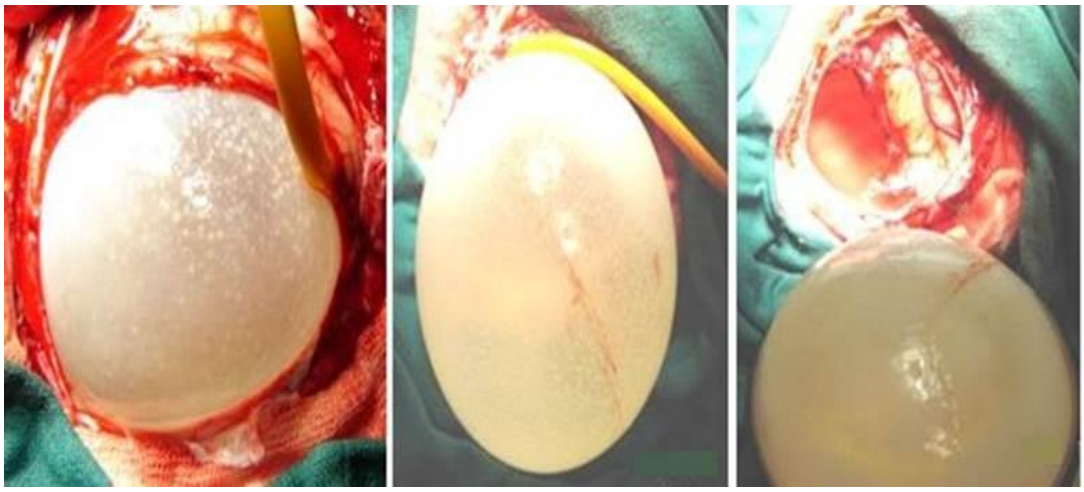

Figure 2.1.5 Dowling technique of solitary cerebral hydatid cyst. Fig 1-"Current Topics in Echinococcosis", book edited by Alfonso J. Rodriguez-Morales, ISBN 978-953-51-2159-6, and Published: September 2, 2015 under CC BY 3.0 license.

The Palanivelu Hydatid System (PHS) helps in the controlled evacuation of the cyst contents, laparoscopically minimizing the fear of intraperitoneal spillage. (Chipde, S.S., Yadav, A., Ranjan, P, .and Kapoor, R., 2012) .

It consists of a 12 mm trocar and cannula assembly along with 5 mm and 8 mm reducers. The pyramidal tip of the trocar has fenestrations and the shaft is hollow to accommodate suction cannula. The 26 cm long cannula has 12 mm inner diameter with suction and irrigation channels [Figure 2]. This unique architecture of trocar and cannula allows for easy suction of scolices, as well as avoids spillage of contents. Following aspiration, the same cannula can be used for visualization of intracystic architecture and rule out its communication with the collecting system. Following the aspiration of the suicidal agent after 10 minutes of contact time, the residual cyst can be marsupial Zed or excised depending on the location. The specimen is retrieved through a specimen retrieval bag. Legend: Palanivelu hydatid system showing the long trocar and cannula with two side channels for suction and irrigation.

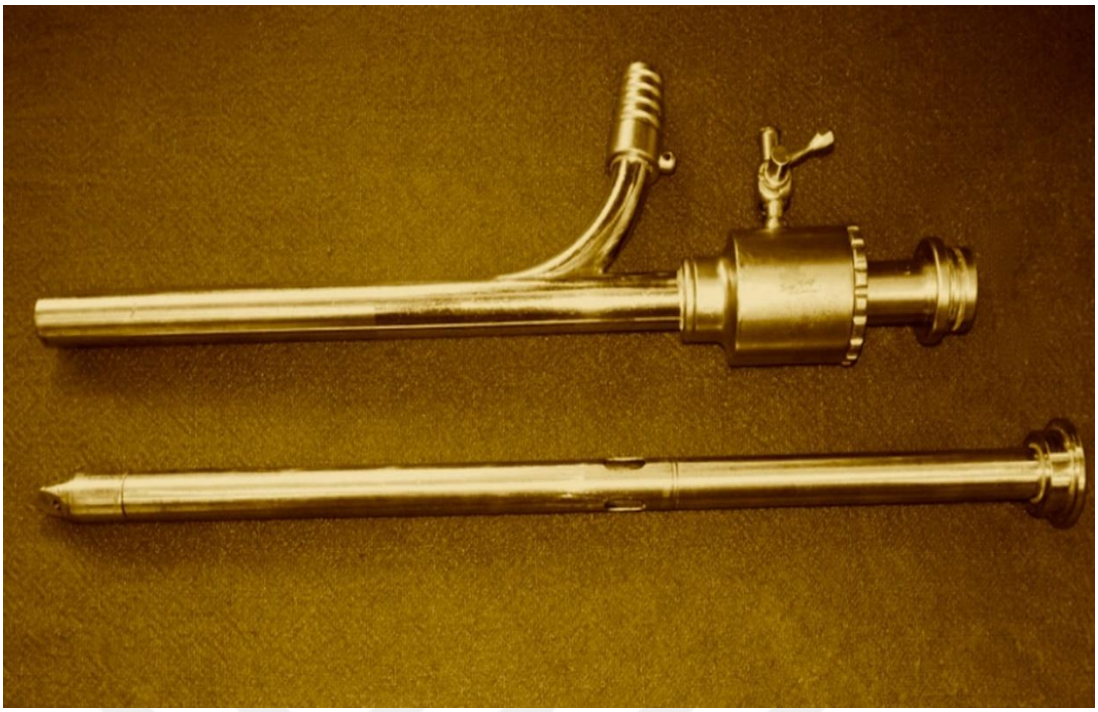

FIGURE- 2.1.6 the Palanivelu Hydatid System (PHS)"Current Topics in Echinococcosis", Book edited by Alfonso J. Rodriguez-Morales, ISBN 978-953-51-2159-6, and Published: September 2, 2015 under CC BY 3.0 license.

Control of unilocular hydatidosis is based on breaking the cycle of infection, either by preventing dogs from consuming infected organs of intermediate hosts or by preventing intermediate hosts from ingesting eggs present in dogs' faces and treating infected dogs with effective cystoids, especially in urban environments. Cystic hydatid disease in humans was found to be caused by different genotypes of E. granules subspecies. Such genotypes include, for example, sense Strict (G1-G3), equines (G4), or Seppi (G5) and Canadensis (G6-G10). (Thompson, R.C.A., 2008).

2.1.7 Prevention and control

1-Prevention of E. granulosus involves meat inspection and destruction of cysts by boiling or burying infected meat to prevent it from being eaten by dogs and other carnivores. The eggs of Echinococcosis are very resistant, to adverse conditions and survive for up to 2 years in water and cool temperatures, and more than a year on the ground under humid conditions. In pure formalin, the eggs remain viable for 2 weeks.

2-Animals should be slaughtered in designated abattoirs where the carcass can be inspected for cysts. Offal from infected animals should be buried or incinerated to prevent dogs from feeding on it.

3-Dogs should be treated regularly with anthelminthic and stray dogs should be eliminated.

4-Human treatment is based on surgical removal of the cyst and\ or treatment. The usual treatment involves albendazole 10 mg/kg body weight. However, success with albendazole is only reported to be about 30%. Administration of praziquantel by itself or in combination with albendazole is 100% effective against the protoscolices.

The species of Echinococcus Speciation in this genus is complex and confusing. Initially, it was considered that there were only one species of Echinococcus granules, although different patterns of development of hydatid cysts were known by parasitologists. These were however thought to be due to development in different intermediate hosts. This view was later refuted and abandoned. Four types of hydatid cysts are now recognized.

Unilocular: characterized by single well-defined bladders, in which the laminated membrane continuously encloses the germinal membrane. Its geographical distribution is global. Example, E. granules.

Multivesicular: characterized by many adjoining and connected bladders, each having its own germinal membrane.

Alveolar: characterized by a malignant type of growth with jelly-filled proliferating vesicles embedded in a common dense stromal. The very thin laminated membrane does not restrict the germinal membrane that grows out into the host surrounding tissues. This parasite is restricted to the northern hemisphere.(Craig, P.S., McManus, D.P.,Lightowlers,M.W.andNieto,A.,2007).

Control of unilocular hydatidosis is based on breaking the cycle of infection, either by preventing dogs from consuming infected organs of intermediate hosts or by preventing intermediate hosts from ingesting eggs present in dogs' faces and treating infected.

Dogs with effective cystoids, especially in urban environments. Cystic hydatid disease in humans was found to be caused by different genotypes of E. granules subspecies. Such Genotypes include, for example, sense Strict (G1-G3), equines (G4), or Seppi (G5) and Canadensis (G6-G10). (Thompson, R.C.A., 2008).

It is most important to use newly developed tools such as imaging, molecular biology, and immunology in both human and animals in any successful control programmer.Moreoveranti-parasitic treatment, control of the definitive hosts, control of slaughtering, vaccination of the intermediate hosts, health education are also considered to be essential elements in any control programmer. (Ito, A., Urbani, C., Jiamin, Q. and Schantz, P.M., 2003).

As it is difficult to completely prevent the exposure to Echinococcus eggs from wild animals, food safety precautions combined with good hygiene can be helpful. All fruits and vegetables, especially those picked up from the wild, should be cleaned thoroughly with water to ensure the removal of the parasite eggs if any. People who handle pets, or are involved in farming, gardening or preparing food, should wash their hands carefully before Eating.

Furthermore, fences should be built around vegetable and fruit gardens to keep dogs and other canids away.

Untreated water from sources such as lakes may also contain Echinococcus eggs and should, therefore, be avoided. Unfortunately, over the past decades, there has been no CE control programmer in Libya, but the high incidence of the disease in humans (1.4 to 2%). (Mohamed, M.I., Wafa, M.I., Kawther, M.I. and Badereddin, B.A., 2017).

Last few years, due to the major social and political changes that affected veterinary and public health services following the collapse of the country government. Conducting screening surveys using serological tests may help in detecting early infections particularly in high-risk groups.

2.2. Heavy metals:

Heavy metals tend to bio accumulate in living creature, and their accumulation has been a major worry. Heavy metals can be qualified as elements that are firmness atomic weight between 63.546 and 200.590 (Kennish, 1991), and are recognized by a specific gravity greater than 4.0 (Connell & Miller, 1984).

Heavy metals are primary components of our environment. They exist in the changeable amount in different ecosystems and continuously cycle through different parts of the biosphere. The advances in human civilization and manufacturing activities have resulted in raising the ratio of various heavy metals in surrounding atmospheres, and this rising may possibly raise further with utilization of geological resources, such as mining and development of fossil fuel and the expanding in the petroleum industry (Aslam, Javed, & Khan, 2011; Grodzińska et al., 2003; Ismail & Beddri, 2009). In spite of that, only tiny quantities of specific heavy metals are required for a number of Biological systems such as copper, chromium and zinc, and some other metals like selenium and vanadium may confer advantageous effects in animals. However, the surplus quantities of these minerals are neatly related with deleterious effects in man and animals (Mohammad et al., 2008; Soetan, Olaiya, & Oyewole, 2010; Valko, Morris, & Cronin, 2005). Their toxicity can result in large health problems, like important body systems like the nervous system lateness in growth and development, cancer, damages to organs and I, and may lead to death, eventually (Akpor & Muchie, 2010). The environmental defilement caused by these metals acts as a serious threat to human health and sustainable development, particularly in pregnancy and later at childhood period, and consequently the development of the ecosystem, in general (Xuexiu, Chuanhao, & Huajiao, 2000).

The adverse effects of known felled metals such as cadmium and lead for their rise toxicity are growing with elevated concentration and their easily uptake by organisms within food chains. The number of heavy metals in soil and water has spectacularly raised starting from the early in the twentieth century as a result of human activities. Therefore; comparing to rural zone, a highly polluted zone with heavy metals are predominately urban areas that are known for their metal-processing actions, which badly affect local population (Valko et al., 2005).

On the rational context, aconcentration of heavy metals in agricultural soils overrides the normal amount and has registered critical scales in some area around the

world. Due to soil contamination by heavy metals, a number of agricultural villages have been abandoned in Eastern Europe, and about 10% of Lands planted with rice in Japan are not suitable for planting anymore (Arao et al., 2010).

Biological or ecological control of heavy metals is an enjoyable and important rapid growing area that composes of utilizing a number of creatures as bio indicators (Rosenberg & Resh, 1993). Fish parasite considered as one of the highly sensitive living organisms to ecological defilement, which can either due to their physiological critical response to contaminants or due to their ability to gather definite toxic substances (Sures, 2004; Vidal-Martinez, Pech, Sures, Purucker, & Poulin, 2010). Heavy metals can be cumulative by a number of intestinal parasites at many times higher focus than those found in the tissues of a host (Sures, 2004).

Some of the Heavy metals can be finished out of the body of humans and other mammals through the bile secreted into the duodenum and finally secrete out of the body with faces Consequently, it's pertinent to investigate the heavy metal bioaccumulation by those parasites inhabiting the biliary branches of mammalian hosts such as liver flukes, particularly of the genus Fasciola. This parasite is one of the well-known pathogens and the first to be discovered and proscribed for being responsible for causing infectious diseases (Lotfy et al., 2008). The Fasciola is a ubiquitous parasite and has a global distribution, by which the infection. Its cause, fascioliasis is very well known in different countries through the world (Mas-Coma, 2004; Mas-Coma, Valero, & Bargues, 2009).

2.2.1 Definitions of a heavy metal

Heavy metals" are chemical elements specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Just expressed, particular gravity is a measure of the thickness of a given measure of a strong substance when it is contrasted with an equivalent measure of water. Some outstanding dangerous metal components with a particular gravity that is at least 5 times that of water the specific gravity mercury, 13.546, lead 11.34 8.65 cadmium; iron, 7.9; lead, arsenic 5.7; (Lide, D.R., 1992. Hdbk of Chemistry & Physics 73rd Edition (Vol. 73). CRC).

2.2.2 Metals and heavy metals

Metals are predominating characterized and featured from nonmetals by their physical properties – the capability to conduct heat, and an electrical resistance that is immediately proportional to temperature, malleability, suppleness and even luster (Appenroth, K.J., 2010). These properties especially that of temperature follower conductivity, at least allow us to define what a metal is in contrast to nonmetals and metalloids. However, as mentioned above, all of these physical properties are missing after the metal has been chemically converted into a chemical compound that can be taken up by plants (Shaw, B.P., and Mishra, R.K., 2004).

It is well known that the properties of chemical elements can be specified from their positions in the periodic table of the elements (Fig. 2.2.1). In public, the chemical elements become more metallic as we transfer towards the lower left corner of the table and nonmetallic towards the top right corner. In other words, metallic character lessening from left to right and from the bottom to the top of the table. Metalloids (elements with properties intermediate between metals and nonmetals) occur shut to the diagonal border between metals and nonmetals in the table. A metal can be categorized according to the last electronic subshell in its atom. There are s-elements, which can be department into alkaline elements (first main group) and alkaline earth elements (second main group). Alls-elements are metals except for H (the first element in the first main group). The first element in the second main group, Be, is also somewhat special (its oxides are amphoteric), but it is still deemed to be a metal. Among the other groups of the periodic table, d-group elements (transition elements) are all metals. Many of them form compounds with various valence states, which is an important factor in their toxicity.

Several of the oxides of transition elements have somewhat amphoteric properties, but they are still all deemed to be metals. Then there are the f-group elements, also known as the scarce earth elements, which are being divided into the lanthanide series (including La) and the actinide series (including Ac). All of these rare earth elements are also metals and so are sometimes called rare earth metals. The following group, the p-group, occurs towards the right-hand side of the periodic table and thus represents a mixed group of metals, metalloids, and nonmetals.

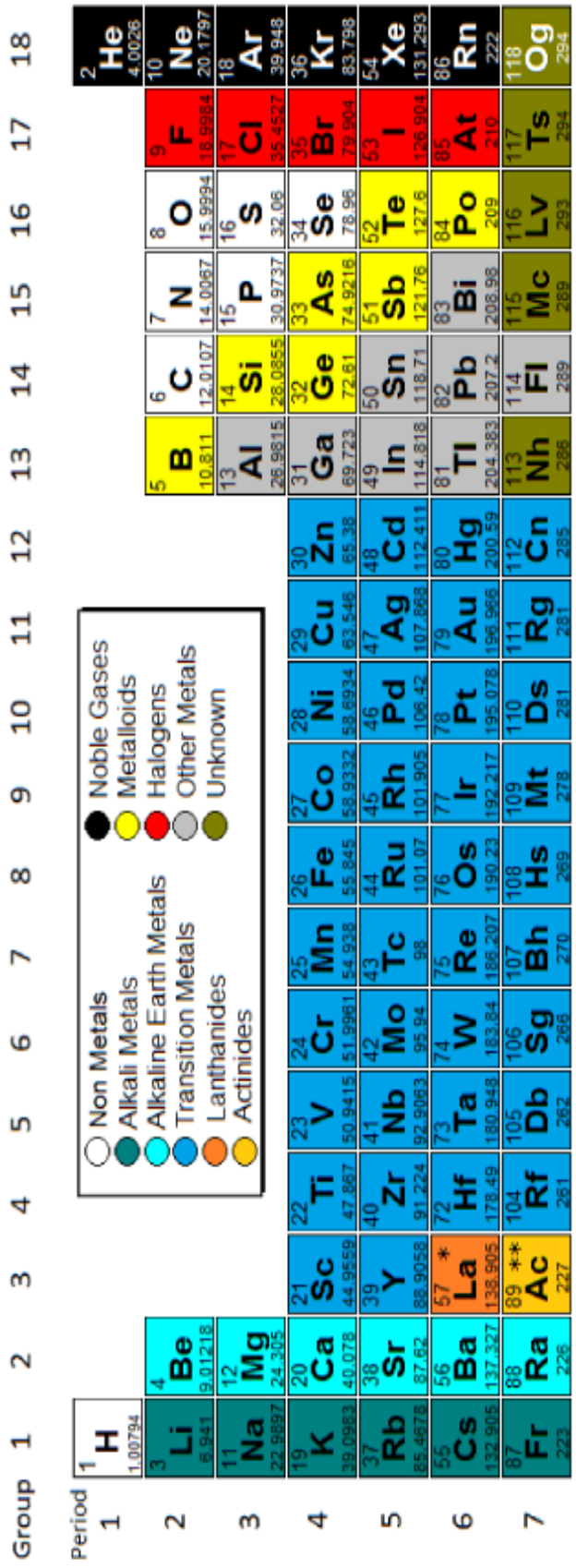

Fig. 2.2.1 Periodic table of the elements. Metals and some metalloids are indicated. The transition Elements, the rare earth elements (lanthanide series, actinide series) and the lead-group elements on the Right hand side of the table are relevant to the definition of "heavy metals".

Metals, metalloids, and non-metals. This includes the elements of the third to seventh main groups of the periodic table but excludes the scarce gases (the eighth main group).

Metallic members of this group include Al, Pb, Cd, Bi, Te, and Ga, In, Tl, Sn, and Po. All of them (except Bi) form amphoteric oxides. Si, Ge, As and Te are considered to be metalloids; on occasion B and Sb are included too (Fig. 2.2.1). Since there is no common name for the metal/metalloid members of the p-group, we suggest that these metals and metalloids should be characterized "lead-group elements", as a leader is the representative of this group that has been studied in the greatest profundity in plant science. As plant scientists, we should stress at this dot that we eternally talk about the elemental forms of these elements. We usually only deal with their salts.

There are, of course, particular cases where the properties of a compound formed from elements from any of the groups defined above are adjusted (e.g. by organic ligands or substituents). This should then be therapy as a special case and does not needs have an impact on the divisions and subdivisions of elements. Classifying metals according to their positions in the periodic table of the elements makes sense because the chemical properties of their compounds are regarding it (Duffus, J.H., 2002).

2.2.3 Toxic heavy metals

Heavy alloy becomes toxic when they are not metabolized by the body and gather in the soft tissue paper. Heavy metal may enter the human being body through food for thought, water, aviation, or absorption through the skin when they come in connect with humans in Department of Agriculture and in industrial, pharmaceutical, industrial, or residential circumstance. Industrial detection Chronicle for a common route of exposure for adults. Ingestion is the most common route of detection in children (Roberts, J.R., 1999). Children may develop toxic levels from the normal deal - to-Rima or activity of small children who come in connect with polluted stain or by actually eating aim that is not food (grime or paint chip shot) (Dupler, D., 2001). Less common road of exposure are during a radiological procedure, from inappropriate dosing or controlling during intravenous (parenteral) nutrition, from a broken thermometer (Smith, J.A., and Sadler, D., 1997), or from a suicide or homicide attempt (Lupton, J.E., Baker, E.T., and Rago, T.A., 1998).

As a dominion, acute toxicity is more probable to effect from inhalation or skin middleman of dust, exhaust or vapours, or materials in the workplace. However, lesser levels of contamination may happen in residential circumstance, especially in older homes with lead paint or old plumbing (Takala, J., 1999) {International Occupational Safety and Health Selective information Heart 1999}.

The representative for Toxic Substances and Disease Registry (ATSDR) in Atlanta, Georgia, (a part of the U.S. Department of Health and Human Services) was established by congressional mandate to carry out specified functions concerning reverse human health effects and diminished quality of life associated with exposure to hazardous substances. The ATSDR is responsible for the assessment of waste sites and providing health information concerning dangerous substances, response to emergency release situations, and education and training concerning dangerous substances (ATSDR Mission Statement, November 7, 2001). In collaboration with the U.S. Environmental Protection representative, the ATSDR has compiled a Priority List for 2001 called the "Top 20 Hazardous Substances." The heavy metals such as arsenic (1), lead (2), mercury (3), and cadmium (7) appear on this list.

As noted earlier, there are 35 metals of concern, with 23 of them called the heavy metals. Toxicity can result from any of these Metals. This protocol will address the metals that are most likely encountered in our daily environment. Briefly covered will be four Metals that are included in the ATSDR's "Top 20 Hazardous Substances" list. Iron and aluminium will also be discussed even though they do not appear on the ATSDR's list.

Lead Pb is number 2 on the ATSDR's "Top twenty Tilt." Lead accounts for most of the cases of paediatric heavy metal poisoning (Roberts, J.R., 1999). Heavy alloys become toxic when they are not metabolized by the body and gather in the soft tissues. It is a very soft metal and was used in pipes, drains, and soldering materials for many geezer hood. Millions of abode built before 1940 still contain jumper lead (e.g., on painted surfaces), leading to chronic exposure from weathering, flaking, chalking, and detritus. Every year, the industry produced about 2.5 one thousand piles of lead throughout the humankind. Most of this lead is used for shelling.

The rest is used for cable natural covering, plumbing, ammunition, and fuel additive.

Other uses are as Paint pigments and in PVC charge plate, x-ray of light shielding, quartz glass production, pencil, and pesticides. Prey organs are the bones,

Einstein, blood, kidney, and thyroid gland (International Occupational Refuge and Health Information Centre 1999; ATSDR ToxFAQs for Lead).

Mercury (Hg) is Number 3 on ATSDR's "Top 20 List" is mercury. Mercury is generated naturally in the environment from the degassing of the earth's crust, from volcanic emissions. It exists in three forms: elemental mercury and organic and inorganic mercury. Mining operations, chloralkali plants, and paper industries are significant producers of mercury (Klaassen, C.D. and Amdur, M.O. eds., 1996). Atmospheric mercury is dispersed across the globe by winds and returns to the earth in rainfall, accumulating in aquatic food chains and fish in lakes (Clarkson, T.W., Friberg, L., and Nylander, M., 1988.).

Mercury compounds were added to paint as a fungicide until 1990. These compounds are now banned; however, old paint supplies and surfaces painted with these old supplies still exist. Mercury continues to be used in thermometers, thermostats, and dental amalgam. (Many researchers suspect dental amalgam as being a possible source of mercury toxicity (Omura, F., Fujita, A1996; O'Brien 2001) Medicines, such as mercurochrome and Merthiolate, are still available. Algaecides and childhood vaccines are also potential sources. Inhalation is the most frequent cause of exposure to mercury. The organic form is readily absorbed in the gastrointestinal tract (90-100%); lesser but still significant amounts of inorganic mercury are absorbed in the gastrointestinal tract (7-15%). Target organs are the brain and kidneys (Patel, S.C., 2010. ATSDR ToxFAQs for Mercury). Cadmium Cd is a by-product of the mining and smelting of lead and zinc and is number 7 on ATSDR's "Top 20 list." It is used in nickel-cadmium batteries, PVC plastics, and paint pigments. It can be found in soils because insecticides, fungicides, sludge, and commercial fertilizers that use cadmium are used in agriculture. Cadmium may be found in reservoirs containing shellfish. Cigarettes also contain cadmium. Lesser-known sources of exposure are dental alloys, electroplating, motor oil, and exhaust.

Inhalation accounts for 15-50% of absorption through the respiratory system; 2-7% of ingested cadmium is absorbed in the gastrointestinal system. Target organs are the liver, placenta, kidneys, lungs, brain, and bones (Patel, S.C., 2010. ATSDR ToxFAQs for Cadmium).

Iron (Iron) does not appear on the ATSDR's "Top 20 List, "but it is a heavy alloy of concern, particularly because ingesting dietary smoothing iron accessory may

acutely poison Loretta Young nestling Ren (e.g.as few as five to nine 30-mg iron tablets for a 30-lb child).

Heavy alloy become toxic when they are not metabolized by the body and gather in the soft tissues. Intake accounts for most of the toxic result of iron because iron is absorbed rapidly in the gastrointestinal tract. The corrosive nature of iron seems to further increment the absorption. Most overdoses appear to be the result of children mistaking Red River -coated ferrous sulphate tablets or grownup multivitamin preparations for confect. In recent years, blister packaging and the requirement that container with 250 mg or more of iron have childproof bottle caps have helped reduce accidental ingestion and overdose of iron tablets by children.) Other sources of iron are drinking water, iron pipes, and cookware. Target organs are the liver, cardiovascular system, and kidney (Henry M. Robert, J.R., 1999).

Although aluminium (Al) is not a heavy metal (specific gravity of 2.55-2.80), it makes up about 8% of the surface of the earth and is the third most abundant element (Patel, S.C., 2010.,ATSDR ToxFAQs for Aluminum).

It is readily available for human ingestion through The use of food additives, antacids, buffered aspirin, astringents, nasal sprays, and antiperspirants; from drinking water; from automobile exhaust and tobacco smoke; and from using aluminium foil, aluminium cookware, cans, ceramics, and fireworks (ATSDR ToxFAQs for Aluminum). Studies began to emerge about 20 years ago suggesting that aluminium might have a possible connection with developing Alzheimer's disease when researchers found what they considered to be significant amounts of aluminium in the brain tissue of Alzheimer's patients. Although aluminium was also found in the brain tissue of people who did not have Alzheimer's disease, recommendations to avoid sources of aluminium received widespread public attention. As a result, many organizations and individuals reached a level of concern that prompted them to dispose of all their aluminium cookware and storage containers and to become wary of other possible sources of aluminium, such as soda cans, personal care Products and even their drinking water. However, the World Health Organization (WHO 2006) concluded that, although there were studies that Demonstrate a positive relationship between aluminium in drinking water and Alzheimer's disease, the WHO had reservations about a causal relationship because the studies did not account for total aluminium intake from all possible sources.

Although there is no conclusive evidence for or against aluminium as a primary cause of Alzheimer's disease, most researchers agree that it is an important factor in the dementia component and most certainly deserves continuing research efforts. Therefore, at this time, reducing exposure to aluminium is a personal decision. Workers in the automobile manufacturing industry also have concerns about long-term exposure to aluminium (contained in metal working fluids) in the workplace and the development of degenerative muscular conditions and cancer. The ATSDR has compiled a ToxFAQs for Aluminum to answer the most frequently asked health questions about aluminium. Target organs for aluminium are the central nervous system, kidney, and digestive system.Arsenic is the most common cause of acute heavy metal poisoning in adults and is number 1 on the ATSDR's "Top 20 List. “Arsenic is released into the environment by the smelting process of copper, zinc, and lead, as well as by the manufacturing of chemicals and glasses. Arsine gas is a common byproduct produced by the manufacturing of pesticides that contain arsenic. Arsenic may be also be found in water supplies worldwide, leading to exposure of shellfish, cod, and haddock.

Other sources are paints, rat poisoning, fungicides, and wood preservatives. Target organs are the blood, kidneys, and central nervous, digestive, and skin systems (Patel, S.C., 2010. ATSDR ToxFAQs for Arsenic).

2.2.4 Beneficial of heavy metals

In small measure, confirmed heavy metallic element s are nutritionally fundamental frequency for a healthy animation. Some of these are referred to as the trace elements (e.g. Zinc. iron, copper, Mn). These elements, or some form of them, is generally found naturally in foodstuff , in fruits and vegetables, and in commercially available multivitamin products (Takala, J., 1999) {International Occupational Refuge and Health Information Pith of attention 1999}. Diagnostic medical applications include direct injection of gallium during radiological procedures, dosing with chromium in parenteral nutrition admixture, and the use of star as a radiation sickness shield around x-ray equipment (Roberts, J.R., 1999). Heavy metals are also common in industrial applications such as in the industry of pesticides, batteries, alloys, electroplated metal parts, textile dyes, steel, and so forth. (Takala, J., 1999) {International Occupational Safety and Health Information Centre 1999}. Many of these products are in our homes and MBD to our quality of life when properly used.

Purpose and aims of the project:

This study is aiming at:

- Evaluate the concentration of heavy metals in worm parasites inhabiting caw, sheep and comparing with their hosts' liver, kidneys and intestinal tissues.
- Evaluating these parasites as bio accumulators of heavy metals.
- Estimate the role of these parasites as sensitive markers of environmental pollution with heavy metals.
- Extract knowledge about the possible undesirable effects of exceed concentration of heavy metals in these consumed animals on the public health.
- Assess the possible protection role of these parasites in protecting their hosts from serious danger through elevate levels of the heavy metals in these parasites rather than the tissues of their hosts.
- Highlight the role of infectious parasites as bio indicators and monitors of the contamination level of heavy metals at both terrestrial and aquatic environments
- Confirm the role of infectious parasites as bio protectors of infected animals by uptake high concentration of toxic metals and therefore reducing the accumulation of these metals in the tissues of infected animals.

3. MATERIAL AND METHOD

3.1. Material

Cylinder, funnel, beaker filter paper watch glass,

Pipet, volumetric flask, conical flask, balance, bottle (250+500) ml

Hot plate, oven, centrifuge,

Hood, gloves, tissues, bio hand (alcohol to cleaning), plastic bags, Blade operations

Para-film, bottle to save solution, falcon tube, pretty-dish, slide and cover, cuter, tongue depressor.

Chemical equipment

Nitric Acid, min.69, 5%, reagent grade, ACS, ISO, max.

Hydrogen peroxide solution

Distell water

Deionized water

Vacuum clever for cleaning materials

Microscope and atomic absorption or ICP.

3.2. Method

The materials used for the study include the field and laboratory materials. The study will be conducted at the laboratories of Koya University/Department of Medical Microbiology.

The present study is conducted to collect specific samples of infected organs (Liver, lung and intestine) of domestic animals that are usually consumed and are infected by helminthic parasites, for the purpose of detecting the concentration of heavy metal in these infected animal tissues. The meat of these animals is an important part of the traditional cuisine of the local population.

The study is designed to involve both terrestrial and aquatic animals to cover a wide range of infected animals in both environments and to give the research more potency and strength. The study will be performed on 100-200 samples from three types of animals (Caw, sheep and fish). Also, around 20 samples will be collected from each type of uninfected animals from the same farms, as a control.

The samples will then be dried and digested with concentrated HNO3 and concentrated H_2O_2. A blank digestion should be prepared out in the same way for the control samples. After digestion, the samples will be allowed to cool down, filtered and then distilled water up to the mark of the volumetric flask. The concentration of heavy

metals in parasitized and digested domestic animal tissues will then be determined using inductively coupled plasma –optical emission spectroscopy

Determination of heavy metals in parasitized liver and lung tissues of terrestrial and aquatic domestic animals by Inductively coupled plasma –optical emission spectroscopy

Procedure

1- Liquid samples (Protoscolices in 15ml falcon tubes), should be centrifuged at (3000rpm) for 10 minutes. Discard the supernatant, and the pellet should be dried by either using oven at 100˚C, or via cooling dry (lyophilisation) by using lyophilizer, until you obtain dry powder samples.

   Tissue samples should be dried by the same way as above, until you obtain dry powder samples.

2- Weight 1 gm. of dried sample, using sensitive balance.

3- Transfer the dried samples into 250ml digestion beaker or flask.

4- Digest the sample by adding 10 ml of concentrated HNO3 and mix well.

5- Heat the digestion mixture on a hot plate at 100 ± 10˚C for 30 min, inside the fume chamber (Hood).

6- Repeat the heating process once more with 10 mL of the acid.

7- Cool down the mixtures to room temperature, then add 2 mL of concentrated H_2O_2.

8- Heat the beaker or flask again carefully, until dryness.

9- Leave to cool down, then dissolve the mixture in distilled or deionized water until obtaining a clear solution. Filter the sample solution through a cellulose filter paper into 25ml digestion tubes.

10- The filtrate was diluted to 25 mL with distilled or deionized water and heated the solution to dissolved the precipitate

11- Transfer the samples into laboratory polyethylene bottles, and store until analysing.

12- A blank digestion should be prepared out in the same way for the control samples.

13- Finally, analyze the heavy metals in the sample solutions by Inductively coupled plasma/optical emission spectroscopy (ICP-OES). The final measurement volume of the sample solutions should be 5 ml.

Inductively coupled plasma –optical emission spectroscopy

Inductively coupled plasma/optical emission spectroscopy (ICP-OES) is a powerful tool for the determination of metals in a variety of different sample matrixes. With this technique, liquid samples are injected into a radiofrequency (RF) induced argon plasma using one of a variety of nebulizers or sample introduction techniques.

The sample mist reaching the plasma is quickly dried, vaporized, and energized through collisional excitation at high temperature.

The atomic emission emanating from the plasma is viewed, collected with a lens or mirror, and imaged onto the entrance slit of a wavelength selection device. Single element measurements can be performed cost effectively with a simple monochromatic/photomultiplier tube (PMT) combination, and simultaneous multielement determinations are performed for up to 70 elements with the combination of a polychromatic and an array detector. The analytical performance of such systems is competitive with most other inorganic analysis techniques, especially with regards to sample throughput and sensitivity (Hou, X., Amais, R.S. and Donate, G.L., 2000).

Principle:

The principle used in the inductively coupled plasma optical emission spectroscopy is when plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the ray's intensity. To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature approximately (8000 °K) and this energy is used in the excitation emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube (Ghosh, S., Prasanna, V.L and Banji, D., 2013).

Inductively Coupled Plasma Characteristics

The main analytical advantages of the ICP over other excitation sources originate from its capability for efficient and reproducible vaporization, atomization, excitation,

and ionization for a wide range of elements in various sample matrices. This is mainly due to the high temperature, 7000 – 8000 K, in the observation zones of the ICP. This temperature is much higher than the maximum temperature of flames or furnaces (3300K). The high temperature

of the ICP also makes it capable of exciting refractory elements, and renders it less prone to matrix interferences. Other electrical-discharge-based sources, such as alternating current and direct current arcs and sparks, and the microwave-induced plasma (MIP), also have high temperatures for excitation and ionization, but the ICP is typically less noisy and better able to handle liquid samples.

In addition, the ICP is an electrode less source, so there is no contamination from the impurities present in an electrode material. Furthermore, it is relatively easy to build an ICP assembly and it is inexpensive, compared to some other sources, such as a laser induced plasma (LIP). The following is a list of some of the most beneficial characteristics of the ICP source.

- high temperature (7000 – 8000 K)
- high electron density (1014–1016 cm3)
- appreciable degree of ionization for many elements
- simultaneous multielement capability (over 70 elements including P and S)
- low background emission, and relatively low chemical interference
- high stability leading to excellent accuracy and precision
- excellent detection limits for most elements (0.1 –100 ng mL-1)
- wide linear dynamic range (LDR) (four to six orders of magnitude)
- Applicable to the refractory elements (Hou, X., Amais, R.S. and Donate, G.L., 2000).

Instrumentation

In inductively coupled plasma-optical emission spectrometry, the sample is usually transported into the instrument as a stream of liquid sample. Inside the instrument, the liquid is converted into an aerosol through a process known as nebulization. The sample aerosol is then transported to the plasma where it is desolated, vaporized, atomized, and excited and/or ionized by the plasma. The excited atoms and ions emit their characteristic radiation which is collected by a device that sorts the

radiation by wavelength. The radiation is detected and turned into electronic signals that are converted into concentration information for the analyst (Ghosh, S., Prasanna, V.L and Banji, D., 2013). A representation of the layout of a typical ICP-OES instrument .

Sample introduction

Liquids are the most common form to be analyzed by plasma emission. These are usually introduced with a nebulizer and spray chamber combination, similar that used for F-AAS. An Aerosol is formed and introduced into the plasma by nebulizer gas stream through the injector tube (Ghosh, S., Prasanna, V.L and Banji, D., 2013).

Nebulizers:

Nebulizers are devices that convert a liquid into an aerosol that can be transported to the plasma. The nebulization process is one of the critical steps in ICP-OES. Commercial instruments uses only two type of mobilizers with an ICP: (i) pneumatic nebulizer and (ii) ultrasonic nebulizer (Hill, S.J., 1993).

Detectors:

Once the proper emission line has been isolated by the spectrometer, the detector and its associated electronics are used to measure the intensity of the emission line. Most commonly used detectors are (Hill, S.J., 1993):

- Photo multiplier tube
- Array detectors
- Photodiode array
- Charge-injection device (CID)
- Charge-coupled device (CCD)

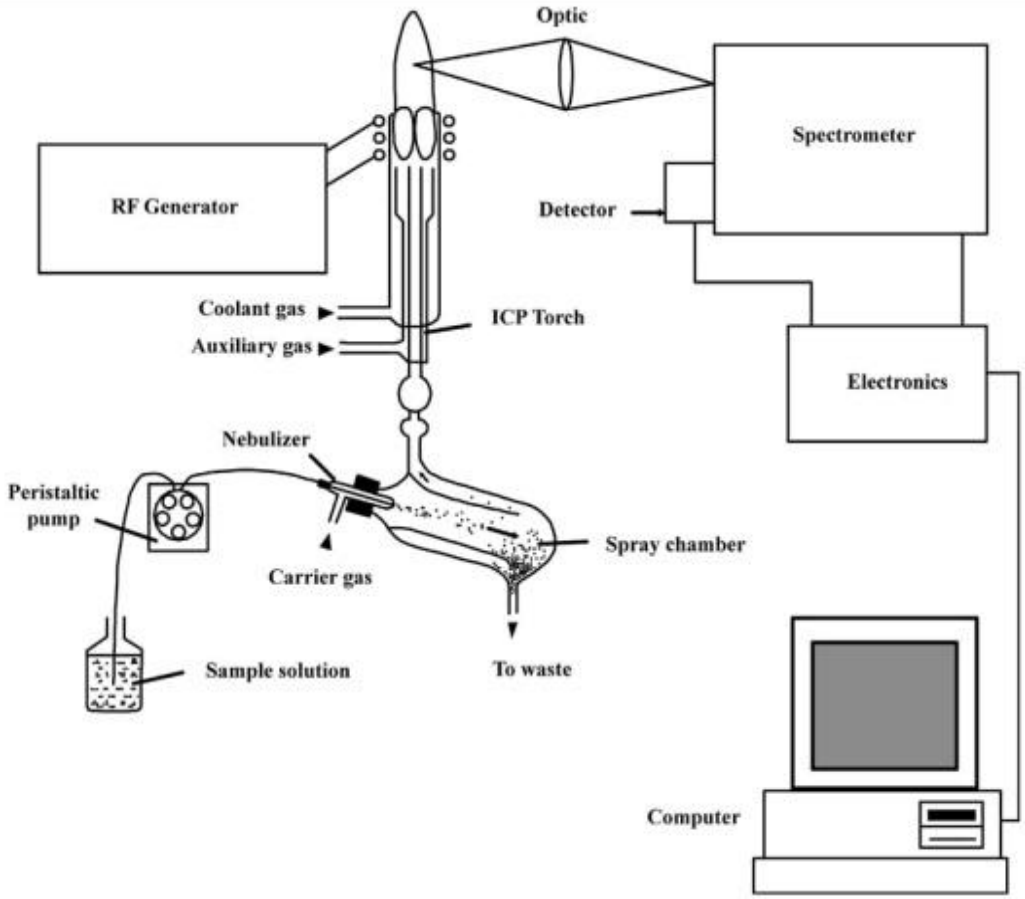

Fig. 3.1. The major components and layout of a typical ICP-OES instrument Ghosh, S., Prasanna, V.L and Banji, D., 2013).

4.1 RESULTS AND DISCUSSION

The study aimed at detect and evaluate the concentration of five heavy metals [Aluminum (Al), Arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb)] in the worm parasite *Echinococcus granulosus* that inhabiting liver and lungs organs of infected domestic animals [Cow (bovine) and sheep], and compare the level of these heavy metals with their hosts' liver and lungs tissues. The parasite was present in the infected tissues in two forms (Hydatid fluid) and (Germinal layer containing protoscolices).

4.1.1. The level of the 5 heavy metals in parasitized Bovine liver Tissue by Echinococcus Granulosus (Hydatid Fluid)

The level of five heavy metals (Al, As, Cd, Hg, and Pb) in infected bovine liver tissues by E. granulosus parasite in form of (Hydatid fluid) was measured. The statistical analysis revealed significant differences between means of studied groups as related with (As, Cd and Pb), which were 11.26, 7.78 and 4.8, ($p<0.05$) for (As), and were 0.33, 0.33 and 2.2, ($p<0.001$) for (Cd), and were 3.44, 3.44 and 4.4, ($p<0.05$) for (Pb), in the hydrated fluid (HF), the infected bovine liver tissue (IBLT) and healthy control, respectively, (Tables 1, 3, 4 and 6).

The results showed no significant differences between means of studied groups as related with (Al and Hg), which were 221.15, 156.9 and 159.8, ($p>0.05$) for (Al), and were 27.1, 26.48 and 25.55, ($p>0.05$) for (Hg), in the hydatid fluid (HF), the infected bovine liver tissue (IBLT) and healthy control, respectively, (Tables 1, 2 and 5).

Table 4.1.1. The level of five heavy metals (Al, As, Cd, Hg and Pb) in infected bovine liver Tissues by E. Granulosus parasite in form of (Hydatid fluid)

		N	Mean	Std. Deviation	P value
Al	Hydatid fluid	7	221.1480	256.45933	0.66
	Infected bovine liver tissue	11	156.8954	125.50611	
	Healthy control	8	159.8115	12.36485	
	Total	26	175.0914	151.46943	
AS	Hydatid Fluid	7	11.2579	4.67146	0.003
	Infected bovine liver tissue	11	7.7865	3.00998	
	Healthy control	8	4.8045	1.44784	
	Total	26	7.8035	3.95824	
Cd	Hydatid Fluid (Parasite)	7	.3330	.00000	0.000
	Infected bovine liver tissue	11	.3330	.00000	
	Healthy control	8	2.1718	.81024	
	Total	26	.8988	.96584	
Hg	Hydatid Fluid	7	27.1107	1.71142	0.300
	Infected bovine liver tissue	11	26.4815	2.35630	
	Healthy control	8	25.5530	1.26829	
	Total	26	26.3652	1.93568	
Pb	Hydatid Fluid	7	3.4470	.00000	0.006
	Infected bovine liver tissue	11	3.4470	.00000	
	Healthy control	8	4.4063	1.14882	
	Total	26	3.7422	.75723	

Table 4.1.2. The level of Aluminum (Al) in infected bovine liver tissues by E. granulose parasite in form of (Hydrated fluid).

Study groups		No.	AL Mean ±SD	P value (ANOVA)
Hydatid Fluid(Parasite)		7	221.148±256.459	P> 0.05
Infected bovine liver tissue		11	156.895±125.506	
Healthy control		8	159.811±12.364	
HF versus IBLT	**LSD**	NS		
HF versus HC		NS		
IBLT versus HC		NS		
HF: hydatid fluid (parasite), IBLT: Infected bovine liver tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb).

Table 4.1.3. The level of Arsenic (As) in infected bovine liver tissues by E. granulosus Parasite in form Of (Hydatid fluid)

Study groups		No.	As Mean ±SD	P value (ANOVA)
Hydatid fluid (Parasite)		7	11.257±4.671	P< 0.05
Infected bovine liver tissue		11	7.786±3.0	
Health control		8	4.804±1.447	
HF versus IBLT	**LSD**	P<0.03		
HF versus HC		P<0.001		
IBLT versus HC		P<0.05		
HF: hydatid fluid (parasite), IBLT: Infected bovine liver tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb).

Table 4.1.4. The level of Cadmium (Cd) in infected bovine liver tissues by E. granulosusparasite in Form of (Hydatid fluid).

Study groups		No.	Cd Mean ±SD	P value (ANOVA)
Hydatid Fluid(Parasite)		7	0.333±0.00	P< 0.001
Infected bovine liver tissue		11	0.333±0.00	
Healthy control		8	2.172±0.810	
HF versus IBLT	**LSD**	NS		
HF versus HC		P<0.001		
IBLT versus HC		P<0.001		
HF: hydatid fluid (Parasite), IBLT: Infected bovine liver tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb).

Table 4.1.5. The level of Mercury (Hg) in infected bovine liver tissues by E. granulosus Parasite in form of (Hydatid fluid)

Study groups		No.	Hg Mean ±SD	P value (ANOVA)
Hydatid fluid (Parasite)		7	27.111±1.711	P>0.05
Infected liver tissue (Bovine)		11	26.481±2.356	
Health control		8	25.553±1.268	
HF versus ILT	**LSD**	NS		
HF versus HC		NS		
ILT versus HC		NS		
HF: Hydatid fluid (Parasite), ILT: Infected liver tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb).

Table 4.1.6. The level of lead (Pb) in infected bovine liver tissues by E. granulosus Parasite in form of (Hydatid fluid).

Study groups		No.	Pb Mean ±SD	P value (ANOVA)
Hydatid fluid (Parasite)		7	3.447±0.00	P< 0.006
Infected liver tissue (Bovine)		11	3.447±0.00	
Health control		8	4.406±1.148	
HF versus ILT	**LSD**	NS		
HF versus HC		P<0.008		
ILT versus HC		P<0.003		
HF: Hydatid fluid (Parasite), ILT: Infected liver tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb).

4.1.2. The level of the 5 heavy metals in parasitized Bovine liver Tissue by Echinococcus Granulosus (Germinal layer)

The level of five heavy metals (Al, As, Cd, Hg, and Pb) in infected bovine liver tissues by E. granulosus parasite in form of (Germinal layer) was also measured. The statistical analysis revealed significant differences between means of studied groups as related with (Cd and Pb), which were 0.33, 0.33 and 2.171, ($p<0.001$) for (Cd), and were 3.48, 3.48 and 4.4, ($p<0.05$) for (Pb), in the Germinal layer (GL), the infected bovine liver tissue (IBLT) and healthy control, respectively, (Tables 7, 10 and 12).

The results showed no significant differences between means of studied groups as related with (Al, As and Hg), which were 247.74, 156.9 and 159.8, ($p>0.05$) for (Al), and were 5.84, 7.78 and 4.8, ($p>0.05$) for (As), and were 25.4, 26.5 and 25.55 ($p>0.05$) for (Hg) in the Germinal layer (GL), the infected bovine liver tissue (IBLT) and healthy control, respectively, (Tables 7, 8, 9 and 11).

Table 4.1.7. The level of the five heavy metals (Al, As, Cd, Hg and Pb) in infected bovine Liver Tissuesby E. granulosus parasite in form of germinal layer (GL)

		N	Mean	Std. Deviation	P value
Al	Liver Bovin GL	10	247.7427	110.76845	
	Liver bovin Tissue	11	156.8954	125.50611	0.098
	Liver Bovin Control	8	159.8115	12.36485	
	Total	29	189.0265	107.18400	
As	Liver Bovin GL	10	5.8418	3.79105	
	Liver bovin Tissue	11	7.7865	3.00998	0.106
	Liver Bovin Control	8	4.8045	1.44784	
	Total	29	6.2933	3.15616	
Cd	Liver Bovin GL	10	.3330	.00000	
	Liver bovin Tissue	11	.3330	.00000	0.000
	Liver Bovin Control	8	2.1718	.81024	
	Total	29	.8402	.92932	
Hg	Liver Bovin GL	10	25.4390	2.19807	
	Liver bovin Tissue	11	26.4815	2.35630	0.461
	Liver Bovin Control	8	25.5530	1.26829	
	Total	29	25.8659	2.04450	
Pb	Liver Bovin GL	10	3.4470	.00000	
	Liver bovin Tissue	11	3.4470	.00000	0.003
	Liver Bovin Control	8	4.4063	1.14882	
	Total	29	3.7116	.72134	

Table 4.1.8. The level of Aluminum (Al) in infected bovine liver tissues by E. granulosus parasite in Form of (Germinal layer).

Study groups		No.	Al Mean ±SD	P value (ANOVA)
Liver Bovine Germinal layer		10	247.742±110.768	P> 0.05
Liver Bovine infected tissue		11	156.895±125.506	
Liver Bovine control		8	159.811±12.364	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*Thelevel of heavy metal was measured in part per billion (ppb).

Table 4.1.9. The level of Arsenic (As) in infected bovine liver tissues by E. granulosus parasite in Form of (Germinal layer).

Study groups		No.	As Mean ±SD	P value (ANOVA)
Liver Bovine Germinal layer		10	5.841±3.791	P> 0.05
Liver Bovine infected tissue		11	7.786±3.00	
Liver Bovine control		8	4.804±1.447	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		NS		
LIT versus HC		P<0.05		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.10. The level of Cadmium (Cd) in infected bovine liver tissues by E. granulosus parasite in Form Of (Germinal layer).

Study groups		No.	Cd	P value
			Mean ±SD	(ANOVA)
Liver Bovine Germinal layer		10	0.333±0.000	P< 0.001
Liver Bovine infected tissue		11	0.333±0.000	
Liver Bovine control		8	2.171±0.810	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		P<0.001		
LIT versus HC		P<0.001		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.11. The level of Mercury (Hg) in infected bovine liver tissues by E. granulosus parasite in Formof (Germinal layer).

Study groups		No.	Hg	P value
			Mean ±SD	(ANOVA)
Liver Bovine Germinal layer		10	25.439±2.198	P> 0.05
Liver Bovine infected tissue		11	26.481±2.356	
Liver Bovine control		8	25.553±1.268	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.12. The level of Lead (Pb) in infected bovine liver tissues by E. granulosus parasite in Form of (Germinal layer).

Study groups		No.	Pb Mean ±SD	P value (ANOVA)
Liver Bovine Germinal layer		10	3.447±0.000	P< 0.05
Liver Bovine infected tissue		11	3.447±0.000	
Liver Bovine control		8	4.406±1.148	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		P< 0.01		
LIT versus HC		P< 0.01		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

4.1.3. The level of The 5 heavy metals in parasitized Sheep liver Tissue by Echinococcus Granulosus (Germinal layer) form

The level of five heavy metals (Al, As, Cd, Hg, and Pb) in infected sheep liver tissues by *E. granulosus* parasite in form of (Germinal layer) was measured. The statistical analysis revealed significant differences between means of studied groups as related with (Pb) only, which was 3.48, 3.48 and 47.7, ($p<0.001$) in the Germinal layer (GL), the infected sheep liver tissue (ISLT) and healthy control, respectively, (Tables 13 and 19).

The results showed no significant differences between means of studied groups as related with the other four metals (Al, As, Cd and Hg), which were 409.74, 537.4 and 318.7, ($p>0.05$) for (Al), and were 7.01, 6.34 and 8.8, ($p>0.05$) for (As), and were 0.7, 0.33 and 2.65 ($p>0.05$) for (Cd), and were 27.27, 26.7 and 25.8 ($p>0.05$) for (Hg) in the Germinal layer (GL), the infected sheep liver tissue (ISLT) and healthy control, respectively, (Tables 13-17).

Table 4.1.13. The level of the five heavy metals (Al, As, Cd, Hg and Pb) in infected sheep liver Tissues by EGranulosus parasite in form of germinal layer (GL).

		N	Mean	Std. Deviation	P value
Al	Liver Sheep GL	11	409.7406	368.52453	
	Infected Liver Sheep Tissue	18	537.3582	848.07847	0.622
	Liver Sheep Control	12	318.7394	216.90447	
	Total	41	439.1334	601.24377	
As	Liver Sheep GL	11	7.0115	4.22029	
	Infected Liver Sheep Tissue	18	6.3472	3.94196	0.283
	Liver Sheep Control	12	8.8023	4.27065	
	Total	41	7.2440	4.14458	
Cd	Liver Sheep GL	11	.7095	1.24886	
	Infected Liver Sheep Tissue	18	.3330	.00000	0.079
	Liver Sheep Control	12	2.6508	4.97017	
	Total	41	1.1124	2.86550	
Hg	Liver Sheep GL	11	27.2768	1.91989	
	Infected Liver Sheep Tissue	18	26.7228	1.80924	
	Liver Sheep Control	12	25.8129	2.32480	0.217
	Total	41	26.6051	2.02917	
Pb	Liver Sheep GL	11	3.4470	.00000	
	Infected Liver Sheep Tissue	18	3.4470	.00000	0.000
	Liver Sheep Control	12	47.7203	41.06584	
	Total	41	16.4050	29.65958	

Table 4.1.14. The level of Aluminum (Al) in infected sheep liver tissues by E. granulosus parasite in Form of (Germinal layer).

Study groups		No.	Al Mean ±SD	P value (ANOVA)
Liver Sheep Germinal layer		11	409.740±368.524	P> 0.05
Liver Sheep infected tissue		18	537.3582±848.078	
Liver Sheep control		12	318.739±216.904	
LSGL vs LIT	**LSD**	NS		
LSGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.15. The level of Arsenic (As) in infected sheep liver tissues by E. granulosus parasite in form Of (Germinal layer).

Study groups		No.	As Mean ±SD	P value (ANOVA)
Liver Sheep Germinal layer		11	7.011±4.220	P> 0.05
Liver Sheep infected tissue		18	6..347±3.941	
Liver Sheep control		12	8.802±4.270	
LSGL vs LIT	**LSD**	NS		
LSGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.16. The level of Cadmium (Cd) in infected sheep liver tissues by E. granulosus parasite In form of (Germinal layer)

Study groups		No.	Cd	P value
			Mean ±SD	(ANOVA)
Liver Sheep Germinal layer		11	0.709±1.248	P> 0.05
Liver Sheep infected tissue		18	0..333±0.000	
Liver Sheep control		12	2.650±4.970	
LSGL vs LIT	**LSD**	NS		
LSGL versus HC		NS		
LIT versus HC		P < 0.05		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.17. The level of Mercury (Hg) in infected sheep liver tissues by E. granulosus parasite in form Of (Germinal layer).

Study groups		No.	Hg	P value
			Mean ±SD	(ANOVA)
Liver Sheep Germinal layer		11	27.276±1.919	P> 0.05
Liver Sheep infected tissue		18	26.722±1.809	
Liver Sheep control		12	25.812±2.324	
LSGL vs LIT	**LSD**	NS		
LSGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.18. The level of Lead (Pb) in infected sheep liver tissues by E. granulosus parasite in form of (Germinal layer).

Study groups		No.	Pb Mean ±SD	P value (ANOVA)
Liver Sheep Germinal layer		11	3.447±0.000	P < 0.001
Liver Sheep infected tissue		18	3.447±0.000	
Liver Sheep control		12	47.72±41.065	
LSGL vs LIT	**LSD**	NS		
LSGL versus HC		P < 0.001		
LIT versus HC		P < 0.001		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

4.1.4. The level of the 5 heavy metals in parasitized Bovine lungs Tissue by Echinococcus Granulosus (Hydatid fluid)

The level of five heavy metals (Al, As, Cd, Hg, and Pb) in infected bovine lungs tissues by *E. granulosus* parasite in form of (Hydatid fluid) was also measured. The statistical analysis revealed significant differences between means of studied groups as related with (Pb) only, which was 12.1, 3.44 and 7.58, ($p<0.05$) in the Hydatid Fluid (HF), the infected bovine lung tissue (IBLT) and healthy control, respectively, (Tables 20 and 25).

results showed no significant differences between means of studied groups as related with the other four metals (Al, As, Cd and Hg), which were 280.2, 222.4 and 118.87, ($p>0.05$) for (Al), and were 9.23, 8.56 and 6.12, ($p>0.05$) for (As), and were 0.82, 0.33 and 0.92 ($p>0.05$) for (Cd), and were 26.76, 26.5 and 25.48 ($p>0.05$) for (Hg) in the Hydatid Fluid (HF), the infected bovine lungs tissue (IBLT) and healthy control, respectively, (Tables 19-23).

Table 4.1.19. The level of the five heavy metals (Al, As, Cd, Hg and Pb) in infected bovine lungs Tissues byE.granulosus parasite in form of Hydatid Fluid (HF).

		N	Mean	Std. Deviation	P value
Al	Lungs Bovin Hydatid Fluid	6	280.2173	216.69693	
	Infected Lung Bovin Tissue	9	222.3724	157.75781	0.189
	Lung Bovin Control	7	118.8797	67.75959	
	Total	22	205.2188	161.83680	
As	Lungs Bovin Hydatid Fluid	6	9.2393	3.64062	
	Infected Lung Bovin Tissue	9	8.5643	3.66798	0.307
	Lung Bovin Control	7	6.1240	4.15218	
	Total	22	7.9720	3.86712	
Cd	Lungs Bovin Hydatid Fluid	6	.8293	.55310	
	Infected Lung Bovin Tissue	9	.3330	.00000	0.107
	Lung Bovin Control	7	.9246	.87624	
	Total	22	.6566	.60790	
Hg	Lungs Bovin Hydatid Fluid	6	26.7630	2.89137	
	Infected Lung Bovin Tissue	9	26.5057	1.70409	0.467
	Lung Bovin Control	7	25.4809	1.32727	
	Total	22	26.2498	1.97496	
Pb	Lungs Bovin Hydatid Fluid	6	12.0680	7.28181	
	Infected Lung Bovin Tissue	9	3.4470	.00000	0.003
	Lung Bovin Control	7	7.5846	3.21683	
	Total	22	7.1147	5.33201	

Table 4.1.20. The level of Aluminum (Al) in infected bovine lungs tissues by E. granulosus parasite in Form Of (Hydatid fluid).

Study groups		No.	Al	P value
			Mean ±SD	(ANOVA)
Lung Bovine Hydatid Fluid		6	280.217±216.696	P> 0.05
Lung Bovine infected tissue		9	222.372±157.757	
Liver Sheep control		7	118.879±67.759	
LBHF vs LIT	**LSD**	NS		
LBHF versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.21. The level of Arsenic (As) in infected bovine lungs tissues by E. granulosus parasite in form Of (Hydatid fluid).

Study groups		No.	As	P value
			Mean ±SD	(ANOVA)
Lung Bovine Hydatid Fluid		6	9.239 ±3.640	P> 0.05
Lung Bovine infected tissue		9	8.564 ±3.667	
Liver Sheep control		7	6.124±4.152	
LBHF vs LIT	**LSD**	NS		
LBHF versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.22. The level of Cadmium (Cd) in infected bovine lungs tissues by E. granulosus parasite in Form Of (Hydatid fluid).

Study groups		No.	Cd	P value (ANOVA)
			Mean ±SD	
Lung Bovine Hydatid Fluid		6	0.829 ±0.553	P> 0.05
Lung Bovine infected tissue		9	0.333 ±0.000	
Liver Sheep control		7	0.924±0.876	
LBHF vs LIT	**LSD**	NS		
LBHF versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.23. The level of Mercury (Hg) in infected bovine lungs tissues by E. granulosus parasite in Form of (Hydatid fluid).

Study groups		No.	Hg	P value (ANOVA)
			Mean ±SD	
Lung Bovine Hydatid Fluid		6	26.763 ±2.891	P> 0.05
Lung Bovine infected tissue		9	26.505 ±1.704	
Lung Sheep control		7	25.480±1.327	
LBHF vs LIT	**LSD**	NS		
LBHF versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.24. The level of Lead (Pb) in infected bovine lungs tissues by E. granulosus parasite in form of (Hydatid fluid).

Study groups		No.	Pb	P value
			Mean ±SD	(ANOVA)
Lung Bovine Hydatid Fluid		6	12.068 ±7.281	P < 0.05
Lung Bovine infected tissue		9	3.447 ±0.000	
Lung Sheep control		7	7.584±3.216	
LBHF vs LIT	**LSD**	P < 0.05		
LBHF versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

4.1.5. The level of the 5 heavy metals in parasitized Bovine lungs Tissue by Echinococcus Granulosus (Germinal Layer)

The level of five heavy metals (Al, As, Cd, Hg, and Pb) in infected bovine lungs tissues by E. granulosus parasite in form of (Germinal layer) was also measured. The statistical analysis showed no significant differences between means of studied groups of all five measured metals (Tables 25 and 30).

Table 4.1.25. The level of the five heavy metals (Al, As, Cd, Hg and Pb) in infected bovine lungs Tissues by E. granulosus parasite in form of germinal layer (GL).

		N	Mean	Std. Deviation	P. value
AL	Lung Bovin GL	4	196.8788	234.54887	
	Infected Lung Tissue Bovin	8	242.7620	155.45664	0.842
	Control Lung Bovin	5	268.0980	176.74262	
	Total	17	239.4177	171.50059	
AS	Lung Bovin GL	4	9.7525	5.28981	
	Infected Lung Tissue Bovin	8	8.5108	3.91747	0.348
	Control Lung Bovin	5	6.0092	2.13496	
	Total	17	8.0672	3.90285	
Cd	Lung Bovin GL	4	.3330	.00000	
	Infected Lung Tissue Bovin	8	.3330	.00000	1.000
	Control Lung Bovin	5	.3330	.00000	
	Total	17	.3330	.00000	
Hg	Lung Bovin GL	4	27.4660	3.51318	
	Infected Lung Tissue Bovin	8	26.2696	1.65701	0.637
	Control Lung Bovin	5	26.3020	1.43299	
	Total	17	26.5606	2.07292	
Pb	Lung Bovin GL	4	3.4470	.00000	
	Infected Lung Tissue Bovin	8	3.4470	.00000	1.000
	Control Lung Bovin	5	3.4470	.00000	
	Total	17	3.4470	.00000	

Table 4.1.26. The level of Aluminum (Al) in infected bovine lungs tissues by E. granulosus parasite in Form of Germinal layer (GL).

Study groups		No.	Al	P value
			Mean ±SD	(ANOVA)
Lung Bovine Germinal layer		4	196.878±234.548	P> 0.05
Lung Bovine infected tissue		8	242.762±155.456	
Lung Bovine control		5	268.098±176.742	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.27. The level of Arsenic (As) in infected bovine lungs tissues by E. granulosus parasite in Form ofGerminal layer (GL).

Study groups		No.	As	P value
			Mean ±SD	(ANOVA)
Lung Bovine Germinal layer		4	9.752 ±5.289	P> 0.05
Lung Bovine infected tissue		8	8.510±3.917	
Lung Bovine control		5	6.009±2.134	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydrated fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.28. The level of Cadmium (Cd) in infected bovine lungs tissues by E. granulosus parasite in Form of Germinal layer (HF).

Study groups		No.	Cd	P value
			Mean ±SD	(ANOVA)
Lung Bovine Germinal layer		4	0.333 ±0.000	P> 0.05
Lung Bovine infected tissue		8	0.333±0.000	
Lung Bovine control		5	0.333±0.000	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.29. The level of Mercury (Hg) in infected bovine lungs tissues by E. granulosus parasite in Form of Germinal layer (GL).

Study groups		No.	Hg	P value
			Mean ±SD	(ANOVA)
Lung Bovine Germinal layer		4	27.466 ±3.513	P> 0.05
Lung Bovine infected tissue		8	26.269±1.657	
Lung Bovine control		5	26.302±1.432	
LBGL vs LIT	**LSD**	NS		
LBGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.30. The level of Lead (Pb) in infected bovine lungs tissues by E. granulosusparasite in form of Germinal layer (GL).

Study groups		No.	Pb	P value
			Mean ±SD	(ANOVA)
Lung Bovine Germinal layer		4	3.447 ±0.000	P> 0.05
Lung Bovine infected tissue		8	3.447±0.000	
Lung Bovine control		5	3.447±0.000	
LBGL vs LIT	**LSD**	P < 0.05		
LBGL versus HC		NS		
LIT versus HC		P < 0.05		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

4.1.6. The level of the 5 heavy metals in Parasitized Sheep lungs tissue by Echinococcus Granulosus (Germinal Layer)

The level of five heavy metals (Al, As, Cd, Hg, and Pb) in infected sheep lungs tissues by E. granulosus parasite in form of (Germinal layer) was also measured. The statistical analysis revealed significant differences between means of studied groups as related with (As and Hg), which were 9.5, 13.98 and 6.0, ($P \leq 0.05$) for (As), and were 27.74, 26.28 and 26.3 ($P \leq 0.05$) for (Hg), in the germinal layer (GL), the infected sheep lung tissue (ISLT) and healthy control, respectively, (Tables 32, 34 and 36).

The results showed no significant differences between means of studied groups as related with the other four metals (Al, Cd and Pb), which were 1443.8, 951.6 and 268.1, ($p>0.05$) for (Al), and were 0.333, 0.333 and 0.333, ($p>0.05$) for (Cd), and were 3.48, 3.48 and 3.48 ($p>0.05$) for (Pb), in the germinal layer (GL), the infected sheep lungs tissue (ISLT) and healthy control, respectively (Tables 31, 32, 34 and 36).

Table 4.1.31. The level of the five heavy metals (Al, As, Cd, Hg and Pb) in infected sheep lungs Tissues by E. granulosus parasite in form of germinal layer (GL)

		N	Mean	Std. Deviation	P. value
Al	Lung Sheep GL	6	1443.8370	1063.15468	
	Infected Lung Sheep GL	8	951.6110	1013.51770	0.129
	Lung Sheep Control	5	268.0980	176.74262	
	Total	19	927.1789	964.51759	
As	Lung Sheep GL	6	9.5085	5.68372	
	Infected Lung Sheep GL	8	13.9830	.00000	0.002
	Lung Sheep Control	5	6.0092	2.13496	
	Total	19	10.4716	4.61595	
Cd	Lung Sheep GL	6	.3330	.00000	
	Infected Lung Sheep GL	8	.3330	.00000	1.000
	Lung Sheep Control	5	.3330	.00000	
	Total	19	.3330	.00000	
Hg	Lung Sheep GL	6	27.7430	1.07463	
	Infected Lung Sheep GL	8	26.2860	.29719	0.024
	Lung Sheep Control	5	26.3020	1.43299	
	Total	19	26.7503	1.13648	
Pb	Lung Sheep GL	6	3.4470	.00000	
	Infected Lung Sheep GL	8	3.4470	.00000	1.000
	Lung Sheep Control	5	3.4470	.00000	
	Total	19	3.4470	.00000	

Table 4.1.32. The level of Aluminum (Al) in infected sheep lungs tissues by E. granulosus parasite in Form of Germinal layer (GL).

Study groups		No.	Al	P value
			Mean ±SD	(ANOVA)
Lung Sheep Germinal layer		6	1443.837±106.154	P> 0.05
Lung Sheep infected tissue		8	951.611±101.517	
Lung Sheep control		5	268.098±176.742	
LSGL vs LIT	**LSD**	NS		
LSGL versus HC		P < 0.05		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.33. The level of Arsenic (As) in infected sheep lungs tissues by E. granulosus parasite In Formof Germinal layer (GL)

Study groups		No.	As	P value
			Mean ±SD	(ANOVA)
Lung Sheep Germinal layer		6	9.508±5.683	P ≤ 0.05
Lung Sheep infected tissue		8	13.983±0.000	
Lung Sheep control		5	6.009±2.134	
LSGL vs LIT	**LSD**	P ≤ 0.05		
LSGL versus HC		NS		
LIT versus HC		P ≤ 0.001		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.34. The level of Cadmium (Cd) in infected sheep lungs tissues by E. granulosus parasite in Form of Germinal layer (GL).

Study groups		No.	Cd	P value
			Mean ±SD	(ANOVA)
Lung Sheep Germinal layer		6	0.333±0.000	P > 0.05
Lung Sheep infected tissue		8	0.333±0.000	
Lung Sheep control		5	0.333±0.000	
LSGL vs LIT	**LSD**	NS		
LSGL versus HC		NS		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.35. The level of mercury (Hg) in infected sheep lungs tissues by E. granulosus Parasite in form of germinal layer (GL).

Study groups		No.	Hg	P value
			Mean ±SD	(ANOVA)
Lung Sheep Germinal layer		6	27.743±1.074	P ≤ 0.05
Lung Sheep infected tissue		8	26.286±0.297	
Lung Sheep control		5	26.302±1.432	
LSGL vs LIT	**LSD**	P ≤ 0.05		
LSGL versus HC		P ≤ 0.05		
LIT versus HC		NS		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

Table 4.1.36. The level of lead (Pb) in infected sheep lungs tissues by E. granulosusparasite In form of germinal layer (GL).

Study groups		No.	Pb	P value
			Mean ±SD	(ANOVA)
Lung Sheep Germinal layer		6	3.447±0.000	P > 0.05
Lung Sheep infected tissue		8	3.447±0.000	
Lung Sheep control		5	3.447±0.000	
LSGL versus LIT	**LSD**	NS		
LSGL versus HC		P ≤ 0.001		
LIT versus HC		P ≤ 0.001		
LBHF: Liver bovine hydatid fluid, LIT: Liver infected tissue, HC: Healthy control				
P<0.001: Highly significant, p<0.05: Significant, NS: Non significant				

*The level of heavy metal was measured in part per billion (ppb)

4.1.7 Comparison of the levels of the 5 heavy metals in parasitized Liver tissues by Echinococcus Granulosus (Germinal layer) Of Both Cow and Sheep

A statistical comparison has been performed between the measured levels of the five heavy metals (Al, As, Cd, Hg, and Pb) in infected liver tissues of both cow and sheep by E. granulosus parasite in form of (Germinal layer), through applying T-test statistical method. The results revealed that only mercury (Hg) exhibited significant differences between means of studied groups, which were 27.34, for parasitized sheep liver, and 25.43, for parasitized bovine liver, as germinal layer ($p \leq 0.05$). While there were no significant differences between means of studied groups as related with other four metals (Al, As, Cd and Hg) (Table 37).

Table 4.1.37. Comparison of the levels of the 5 heavy metals in parasitized liver tissues By E. granulosus (Germinal layer) of cow and sheep, applying T-test.

Heavy metals	Groups	N	Mean	Std. Deviation	P value
Al	Liver Sheep GL	10	431.4203	380.99321	0.172
	Liver Bovin GL	10	247.7427	110.76845	
As	Liver Sheep GL	10	7.4215	4.21127	0.390
	Liver Bovin GL	10	5.8418	3.79105	
Cd	Liver Sheep GL	10	.7472	1.30982	0.343
	Liver Bovin GL	10	.3330	.00000	
Hg	Liver Sheep GL	10	27.3455	2.00945	0.058
	Liver Bovin GL	10	25.4390	2.19807	
Pb	Liver Sheep GL	10	3.4470	.00000	1.000
	Liver Bovin GL	10	3.4470	.00000	

4.1.8 Comparison of the levels of the 5 heavy metals in Parasitized Lungs Tissues by E. Granulosus (Germinal layer) of both Cow and Sheep

A statistical comparison has been performed between the measured levels of the five heavy metals (Al, As, Cd, Hg, and Pb) in infected lungs tissues of both sheep and bovine by *E*. granulosus parasite in form of (Germinal layer), through applying T-test statistical method. The results revealed that Aluminum (Al), cadmium (Cd) and lead (Pb) exhibited significant differences between means of studied groups, which were 1443.8ppb, 196.87ppb for (Al)($p \leq 0.05$), 0.333ppb,0.333ppb, for (Cd)($p \leq 0.05$), and 3.48ppb,3.48ppb, for (Pb)($p \leq 0.05$), for parasitized sheep lungs and parasitized bovine lungs, respectively. While there was no significant differences between means of studied groups as related with the other two metals (As and Hg) (Table 38).

Table 4.1.38. Comparison of the levels of the 5 heavy metals in parasitized lungs tissues by E. Granulosus (Germinal layer) of both cow and sheep.

	Groups	N	Mean	Std. Deviation	P value
Al	Lung Sheep GL	6	1443.8370	1063.15468	0.034
	Lung Bovin GL	4	196.8788	234.54887	
As	Lung Sheep GL	6	9.5085	5.68372	0.947
	Lung Bovin GL	4	9.7525	5.28981	
Cd	Lung Sheep GL	6	.3330	.00000	0.018
	Lung Bovin GL	4	.3330	.00000	
Hg	Lung Sheep GL	6	27.7430	1.07463	0.857
	Lung Bovin GL	4	27.4660	3.51318	
Pb	Lung Sheep GL	6	3.4470	.00000	0.018
	Lung Bovin GL	4	3.4470	.00000	

4.1.9. Comparison of the levels of the 5 heavy metals in Parasitized Bovine Liver and Lungs by E. Granulosus (Germinal layer)

A statistical comparison has been performed between the measured levels of the five heavy metals (Al, As, Cd, Hg, and Pb) in infected bovine liver and lungs tissues by *E*. granulosus parasite in form of (Germinal layer), through applying T-test statistical method. The results revealed that only cadmium (Cd) exhibited significant differences between means of studied groups, which was 0.333ppb, 0.333ppb ($p \leq 0.001$), for parasitized bovine liver and lungs, respectively. While there was no significant differences between means of studied groups as related with the other four metals (Al, As, Cd and Hg) (Table 39).

Table 4.1.39. Comparison of the levels of the 5 heavy metals in parasitized bovine liver And lungs tissues by E. granulosus (Germinal layer).

	Groups	N	Mean	Std. Deviation	P value
Al	Liver Bovin GL	10	247.7427	110.76845	0.581
	Lung Bovin GL	4	196.8788	234.54887	
As	Liver Bovin GL	10	5.8418	3.79105	0.143
	Lung Bovin GL	4	9.7525	5.28981	
Cd	Liver Bovin GL	10	.3330	.00000	0.001
	Lung Bovin GL	4	.3330	.00000	
Hg	Liver Bovin GL	10	25.4390	2.19807	0.211
	Lung Bovin GL	4	27.4660	3.51318	
Pb	Liver Bovin GL	10	3.4470	.00000	0.254
	Lung Bovin GL	4	3.4470	.00000	

4.1.10. Comparison of the levels of the 5 heavy Metals in Parasitized Liver and Lungs Tissues of Sheep by E.granulosus (Germinal layer)

A statistical comparison has been performed between the measured levels of the five heavy metals (Al, As, Cd, Hg, and Pb) in infected liver and lungs tissues of sheep by E. granulosus parasite in form of (Germinal layer), through applying T-test statistical method.

The results revealed that only lead (Pb) exhibited significant differences between means of studied groups, which were 3.48, for parasitized sheep liver, and 3.48, for parasitized sheep lungs ($p \leq 0.05$). While there were no significant differences between means of studied groups as related with the other four metals (Al, As, Cd and Hg) (Table 40).

Table 4.1.40. Comparison of the levels of the 5 heavy metals in parasitized liver and lungs tissues Of Sheep by E. granulosus (Germinal layer), by T-test.

Groups		N	Mean	Std. Deviation	P value
Al	Liver Sheep GL	11	409.7406	368.52453	0.063
	Lung Sheep GL	6	1443.8370	1063.15468	
As	Liver Sheep GL	11	7.0115	4.22029	0.318
	Lung Sheep GL	6	9.5085	5.68372	
Cd	Liver Sheep GL	11	.7095	1.24886	0.478
	Lung Sheep GL	6	.3330	.00000	
Hg	Liver Sheep GL	11	27.2768	1.91989	0.594
	Lung Sheep GL	6	27.7430	1.07463	
Pb	Liver Sheep GL	11	3.4470	.00000	0.002
	Lung Sheep GL	6	3.4470	.00000	

4.2. Analytical figures of merits

In order to establish the calibration a set of multi-element standards from 0.1 mg/L to 13mg/L was prepared and the intensities registered then evaluated three proceduresabout of DL, QL, sensitivity, line range, reproducibility and correction coefficient.

The analytical parameters of the calibration curves are showed in table (3.7) obtained by ICP-OES. The (DL) and (QL) were calculated in µg/g unit

According to equation (3.3), (3.4) DL and QL calculated for ICP-OES.

Equation (**Hata! Belgede belirtilen stilde metne rastlanmadı.**.1): The DL Calculated in µg/g unit for ICP-OES

$$3\ BEC\ *\ RSD\ blk \qquad (3.3)$$

Equation (**Hata! Belgede belirtilen stilde metne rastlanmadı.**.2) The QL Calculated in µg/g unit for ICP-OES

$$10\ BEC\ *\ RSD\ blk \qquad (3.3)$$

BEC= Background equivalence correction and RSD are relative standard deviation of blank.

Table 4.2.1. Characteristics data of the calibration curves of elements using ICP-OES

Elements	Equation	R^2	Wave length (nm)	Linear range ($\mu g.L^{-1}$)	DL ($\mu g.L^{-1}$)	QL ($\mu g.L^{-1}$)
Be	y = 4999.4x - 4749.5	0.99996	313.042	0.607-4200	0.607	2.0
Ce	Y= 47.885x + 59239	0.99996	418.660	48.28-12000	48.28	160.9
Cu	y = 141.57x + 21244	0.99997	324.754	3.925-12000	3.925	13.1
Fe	y = 85.595x + 9388.4	0.99999	259.941	6.923-12000	6.923	23.1
K	y = 4.9995x + 15808	0.99993	766.491	135.5-11760	135.5	451.6
Li	y = 221.2x + 27995	0.99997	670.780	4.350-2400	4.350	14.5
Mn	y = 442.24x + 10479	0.99995	257.611	0.711-6000	0.711	2.4
Mo	y = 29245x + 3929.8	0.99994	202.095	4.829-6000	4.829	16.1
Na	y = 50.901x + 55018	0.99937	589.592	41.63-6000	41.63	138.8
Ni	y = 0.9998x + 0.2925	0.99995	231.604	6.424-11760	6.424	21.4
Ca	y = 64.213x + 1659.8	0.99999	396.847	6.960-13200	6.960	23.2
V	y = 37.682x + 8470.6	0.99996	292.464	7.323-12120	7.323	24.4
Zr	y = 281.98x + 28774	0.99997	339.198	5.028-6000	5.028	16.8
Ti	y = 478.27x + 17315	0.99997	334.941	2.251-4500	2.251	7.5
Zn	y = 199.98x + 74.4	0.99999	213.656	1.089-6000	1.089	3.6
Pb	y = 15.105x + 6200.6	0.99979	220.353	2.140-10000	2.140	7.1
Cd	y = 199.99x + 67.5	0.99999	214.438	12.04-10000	12.04	40.1
As	y = 12.441x + 2508.5	0.99991	189.042	51.01-5000	51.01	170.0
Cr	y = 12.37x + 3440	0.99995	267.716	15.04-5000	15.04	50.1

5-CONCLUSION AND RECOMMENDATION

The results of the present study indicate that Echinococcus cestode parasites are useful markers of environmental pollution with some heavy metals such as Aluminum (Al), Arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb). Also, the present results suggest usage of cestode worms in assessment of the environmental deterioration by such metals.

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