Extraction and Estimation of Some Trace Elements from Different Fish Species from Tigris River, Kurdistan Region of Iraq

Arkan Fouad Radeef*, Azzam Abdulsattar Mosa and Raeed Megeed Qadir
Department of Chemistry, Faculty of Science, University of Duhok, Duhok, Kurdistan Region, Iraq
Email: arkanfouad@yahoo.com
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Abstract
The objective of the current study involved the description of an important method of extracting some trace elements such as Calcium (Ca), Iron (Fe), Zinc (Zn), Copper (Cu), Manganese (Mn), Magnesium (Mg), Sodium (Na) and Potassium (K) from four fish species; Crucian Carp *(Carassius carassius),* Asian Jirri (*Silurus triostegus*), Himri (*Barbas luteus*) and Marmaritch (*Mastacembleus mastacembleus*) by using a mixture of strong acids in the edible muscle tissues of these fish species from Tigris river (Kurdistan Region, Duhok-Sharea). The minerals were measured by atomic absorption spectrophotometer. The chemical analyses revealed that the concentrations of some trace elements in fishes were significantly different among fish species. Concentrations of Ca, Fe and Na were significantly higher in muscle of Himri ($p< 0.001$), whereas Cu and K were significantly higher in Crucian Carp ($p< 0.001$). Asian Jirri has the highest levels ($p< 0.001$) of Zn. On the other hand, no significant differences were observed in Mn and Mg levels between fish species.

Keywords: Fish, Trace elements, Atomic absorption.

Introduction:-
Fish is a very important part of a healthy diet. There are many studies on the nutritive value of fish meat, not because of the high protein digestibility but also because of omega-3 fatty acids, mineral matter and vitamins content [1, 2]. Commercial aqua feed has been traditionally based on fish meal as a main protein source due to its high protein content and balanced amino acid profile. In addition, it is considered as an excellent source of essential fatty acids, minerals and vitamins [3]. Fish meal is also very palatable and highly digestible to most fresh water and marine fishes [4]. Therefore, fish should be an integral component of the diet, preventing malnutrition by making these macro and micro nutrients readily available to the body. Fish's proteins are important for growth and development of the body, maintenance and repairing of worn out tissues and for production of enzymes and hormones required for many biological processes [5]. The importance of fish in providing easily digested protein of high biological value is well documented. On a fresh weight basis, fish contains about 18-20% protein and contains all the essential amino acids including the sulfur containing lysine, methionine and cystein [6]. The fat content of fish varies depending on the species as well as the season but, in general, fish have less fat than red meat. The fat content ranges from 0.2% to 25%. However, fats from fatty fish species contain the polyunsaturated fatty acids (PUFAs) namely EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) (Omega-3-fatty acids) which are essential for proper growth of children and protect the body from the risks of cardiovascular diseases such a coronary heart disease. In pregnant women, the presence of PUFAs in their diets has been associated with proper brain development among unborn babies [6]. In other studies, omega-3-fatty acids have also been associated with reduced risk of preterm delivery and low birth weight. The fat also contributes to energy supplies and assists in the proper absorption of fat soluble vitamins namely A, D, E, and K [6]. The health benefits of fish oil include its ability to aid in treatment of heart diseases, high cholesterol, depression, anxiety, low immunity, cancer, diabetes, inflammation, arthritis, AIDS, Alzheimer's disease, eye disorders, macular degeneration and ulcers. It also helps in weight loss, pregnancy, fertility and skin care; particular for disorders such as psoriasis, acne [7]. The minerals present in fish include iron, calcium, zinc, iodine, phosphorus, selenium and fluorine. These minerals are highly bioavailable and easily absorbed by the body [6].

The aim of the current study was the description of a simple method for extraction of eight trace elements from the muscle of four different fish species, namely Crucian Carp (*Carassius carassius*), Asian Jirri (*Silurus triostegus*), Himri (*Barbas luteus*) and Marmaritch *(Mastacembleus mastacembleus*) which are taken from Tigris river (Kurdistan Region, Duhok-Sharea) using a mixture of strong HNO_3 and $HClO_4$ and the determination of the concentrations of these trace elements in the muscles of these fish species.

Materials and methods:-
Preparation of samples: All types fish species used in the current study were collected during January 2010 from Tigris River (Sharea-Duhok, Kurdistan Region-Iraq). Five individuals from each fish species were used. The fish species used were: Crucian Carp *(Carassius carassius)*, Marmaritch *(Mastacembleus mastacembleus)*, Himri *(Barbas luteus)* and Asian Jirri *(Silurus triostegus).* Muscle's samples were taken from the fish body and stored at – 20 ºC.

Extraction method: Five grams dry weight of fish's muscles was placed into a digestion tube. Five ml of concentrated HNO_3 and concentrated 5 ml of $HClO_4$ were added to the samples, respectively. The reaction has been allowed to proceed, then the reaction was slowed down by placing the tubes in a hot-block

digestion apparatus and heated up to 60 ºC for 30 min. After that the tubes were removed from the hot-block and allowed to cool down, then 10 ml of concentrated HNO_3 was added for each sample and the tubes were returned to digestion rack, heated slowly to 120 ºC and then to 150 ºC. The tubes were removed when samples became black. After cooling, 1 ml H_2O_2 was added. A vigorous reaction has been occurred. The tubes were returned to the block. The H_2O_2 additions were repeated until the samples were clear. Finally the tubes were removed and diluted with deionized water to 50 ml in volumetric flasks [8].

Measurements: Concentrations of Ca, Fe, Zn, Cu, Mn, Mg, Na and K were measured using Atomic Absorption Spectrophotometer (A Analyst 200, PerkinElmer Instruments LLC, USA, 2002) according to the manufacturer's recommendation where original standards were used for the calibration curve for each element. The Blank solution was treated similar to the samples and containing the same amount of acids.

Statistical Analysis: The statistical analysis carried out by using the computer program graph pad prism. Newman-keuls's statistical method was used to compare between the fish species where Mean, Standard error and Analysis of variance (ANOVA) test in the level of ($p<0.001$) were preformed.

Results and Discussions:-

The mean weights and lengths of these species were: 348±10.2 g and 23±2.1 cm *(Crucian Carp)*, 258±8.9 g and 48.7±6.8 cm *(Marmaritch)*, 232±7 g and 31.7±1.7 cm *(Himri)* and 848±13.6 g and 46±3 cm *(Asian Jirri)*.

The mean concentrations of Ca, Fe, Zn, Cu, Mn, Mg, Na and K in muscles of four fish species Crucian Carp *(Carassius carassius)* (C), Marmaritch *(Mastacembleus mastacembleus)* (M), Himri *(Barbas luteus)* (H) and Asian Jirri *(Silurus triostegus)* (J) are listed in figures (1), (2), (3), (4), (5), (6), (7) and (8).

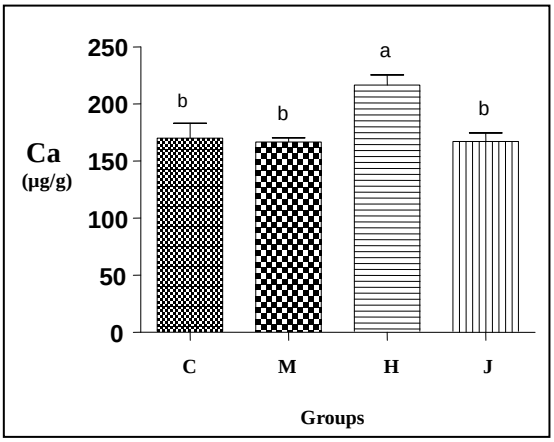

Figure (1): Mean concentrations of Ca in muscles of four fish species Crucian Carp (C), Marmaritch (M), Himri (H) and Asian Jirri (J).

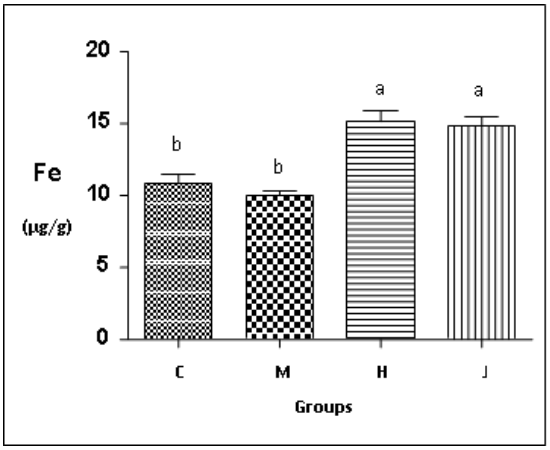

Figure (2): Mean concentrations of Fe in muscles of four fish species Crucian Carp (C), Marmaritch (M), Himri (H) and Asian Jirri (J).

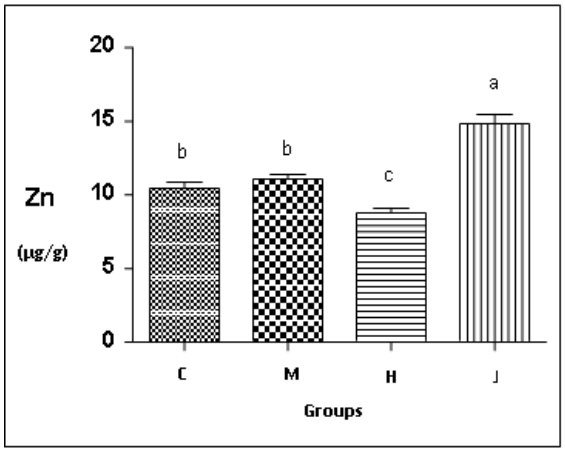

Figure (3): Mean concentrations of Zn in muscles of four fish species Crucian Carp (C), Marmaritch (M), Himri (H) and Asian Jirri (J).

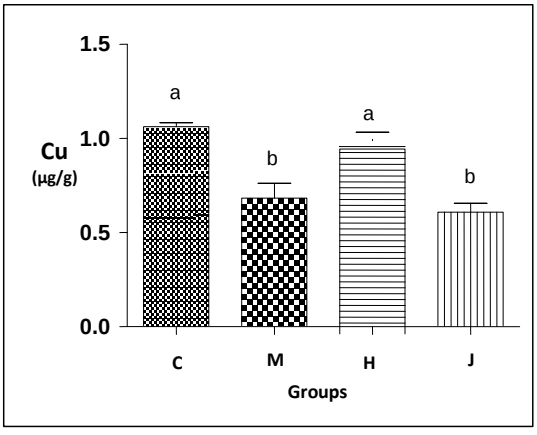

Figure (4): Meanconcentrations of Cu in muscles of four fish species Crucian Carp (C), Marmaritch (M), Himri (H) and Asian Jirri (J).

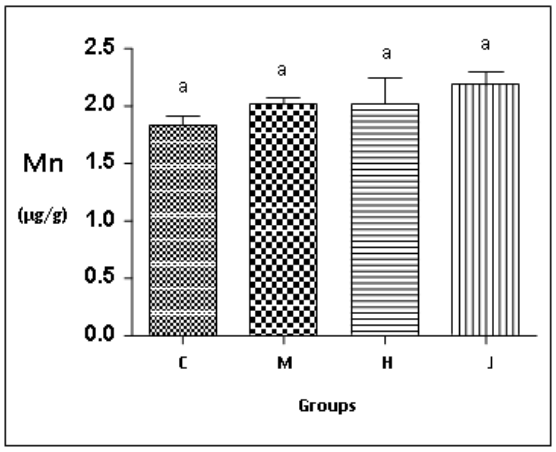

Figure (5): Mean concentrations of Mn in muscles of four fish species Crucian Carp (C), Marmaritch (M), Himri (H) and Asian Jirri (J).

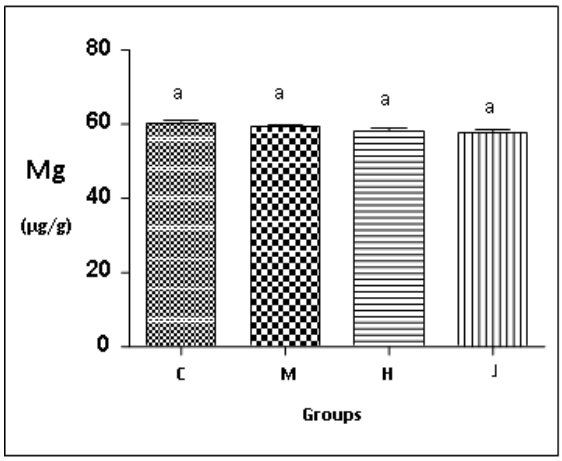

Figure (6): Mean concentrations of Mg in muscles of four fish species Crucian Carp (C), Marmaritch (M), Himri (H) and Asian Jirri (J).

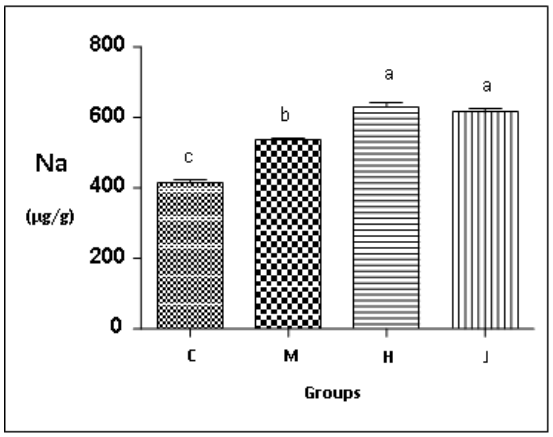

Figure (7): Mean concentrations of Na in muscles of four fish species Crucian Carp (C), Marmaritch (M), Himri (H) and Asian Jirri (J).

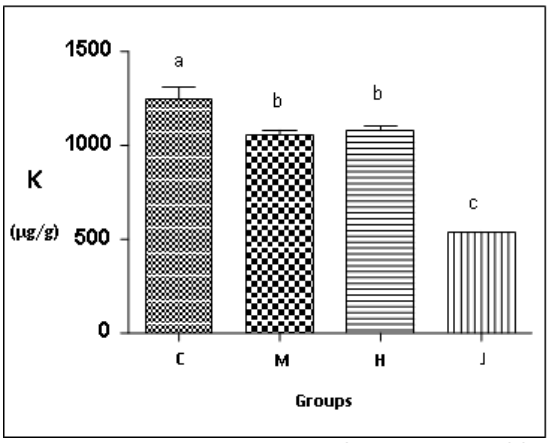

Figure (8): Mean concentrations of K in muscles of four fish species Crucian Carp (C), Marmaritch (M), Himri (H) and Asian Jirri (J).

The obtained results indicate that the highest concentration of calcium (Ca) found in the Himri fish which samples was 216.5±9.03 µg/g ($p<0.001$), while the concentration of calcium (Ca) in the other species were found to be less with $p>0.001$. The highest levels of Iron (Fe) was found also in Himri fish type samples 15.15±0.70 µg/g with no significant difference with Asian Jirri ($p>0.001$) and significant difference with Crucian Carp and Marmaritch with ($p<0.001$), respectively.

Zinc (Zn) levels were significantly highest in Asian Jirri, with an average value of 14.84±0.64 µg/g. There was no significant difference between Crucian and Marmaritch. Zinc (Zn) levels were significantly lowest in Himri, with an average value of 8.78±0.35 µg/g. The highest level concentration of copper (Cu) was observed in Crucian Carp (1.06±0.02 µg/g) with no significant difference with Himri ($p>0.001$) and significant difference with Asian Jirri and Marmaritch with ($p<0.001$), respectively.

There was no significant difference observed in manganese (Mn) levels among the four different types of fish with $p>0.001$. Magnesium (Mg) levels was also have no significant difference in the four different fish types with $p>0.001$.

Himri contains the higher level of sodium (Na) (630.5±3.34 µg/g) compared with Asian Jirri and with Marmaritch, Crucian Carp with $p<0.001$. The higher K concentration was found in Crucian Carp 1249±60.62 µg/g, with $p<0.001$, while the lower K concentrations was found in Asian Jirri 534.7±5.23 µg/g.

Knowledge of metal concentrations in fish muscle is important both with respect to nature management and human consumption of fish and to determine the most useful bio monitor species for human health.

The observed differences can be explained by the fact that, the concentrations of these metals depend to a great extent on species, sex, biological cycle, and on the part of the fish analyzed [9]. Moreover, ecological factors such as season, location/environment of development, nutrient availability, temperature and salinity of the water, may contribute to variations in

the metal concentrations in fishes. The variability in metal levels in different species depends on feeding habits [10]; ecological needs and metabolism [11]; age, size and length of the fish and fish habitat [12, 13].

Western Australian Food and Drink Regulations recommended a level of 40µg/g Zn for human consumption [14]. Accordingly, the concentrations of Zn in the muscles of the studied fish are still below the permissible level. The concentrations of Cu in the muscles of the studied fish are still below the permissible level for Cu (30µg/g) recommended by the National Health and Medical Research Council [14].

Conclusion:-

It can be concluded that concentrations of Ca, Fe and Na were significantly higher in muscle of Himri ($p< 0.001$), whereas Cu and K were significantly higher in Crucian Carp ($p< 0.001$). Asian Jirri has the highest levels ($p< 0.001$) of Zn. On the other hand, no significant differences were observed in Mn and Mg levels between the four fish species.

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استخلاص وتقدير بعض العناصر النزره من انواع اسماك مختلفة من نهر دجلة في كردستان العراق

أركان فؤاد رديف ، عزام عبد الستار موسى ، رائد مجيد قادر

قسم الكيمياء ، كلية العلوم ، جامعة دهوك ، دهوك ، العراق

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الملخص

تضمنت الدراسة الحالية وصف طريقة أستخلاص بعض العناصر النادرة من اربعة انواع مختلفة من الاسماك وذلك بأستخدام مزيج من الحوامض المركزة، كذلك تم تقدير مستويات هذه العناصر والتي تضمنت (الكالسيوم والحديد والخارصين والنحاس والمنغنيز والمغنيسيوم والصوديوم والبوتاسيوم) في الانسجة العضلية الصالحة للأكل في بعض انواع الاسماك (الكارب والجري والحمراوي والمرمريج) المأخوذه من نهر دجلة في منطقة شاريه في محافظة دهوك، اقليم كردستان، العراق. وقد تم تقدير العناصر المذكوره بأستخدام تقنية امتصاص الطيف الذري اللهبي حيث اشارت التحليلات الكيميائية ان مستويات بعض العناصر في انواع الاسماك الاربعة كانت مختلفة، فوجد تراكيز الكاليسيوم والحديد والصوديوم كانت عاليه في الانسجة العضلية لسمك الحمراوي ($p<0.001$)، اما تراكيز النحاس والبوتاسيوم فكانت اعلى في الانسجة العضلية لسمك الكارب ($p<0.001$) ، ووجد بالنسبه الى سمك الجري فأنه يمتلك اعلى نسبة من الخارصين في انسجتة العضلية مقارنة مع الانواع الاخرى من الأسماك ($p<0.001$) ، ولا توجد اي فروقات معنويه يمكن ملاحظتها في مستوى كل من المغنيسيوم والمنغنيز بين انواع الاسماك الاربعة.