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RESEARCH ARTICLE

Chemometric Classification of Some Elements in Wild and Farmed Bluefin Tuna (*Thunnus thynnus* L1758)

Ozlem SOGUT * Fatih PERCIN ** Sibel KONYALIOGLU ***

* Ege University, Faculty of Pharmacy, Analytical Chemistry Department, TR-35100 Bornova, Izmir - TURKEY
** Ege University, Faculty of Fisheries, TR-35100 Bornova, Izmir - TURKEY
*** Ege University, Faculty of Pharmacy, Biochemistry Department, TR-35100 Bornova, Izmir - TURKEY

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Summary

Bluefin tuna *Thnunnus thynnus* 1758 (BFT) belongs to Scombridae family, is native species to subtropical regions of Atlantic Ocean, Mediterranean and Black Sea. The concentration some essential elements (Cu, Fe, Mg, Mn, Ni, Zn) and non-essential element (Pb) have been determined a total of 60 individuals of *T. thynnus* were collected: 30 captured in the wild and 30 sampled from a tuna farm using flame atomic absorption spectrometry. A preliminary chemometric study with the use of patttern recognition methods was carried out in order to characterise, classify and distinguish the different parts BFT. Multivariate chemometric techniques such as principle component analysis (PCA) and K nearest neighbour (KNN) are used to classify BFT to their type and origin on the basis of chemical data. Metal concentrations seem to be good candidates for classification system, as they are stable. This study clearly reported that PCA procedures appear useful tools for differentiation and classification of wild and farmed BFT using the profile of trace elements.

Keywords: *Trace metals, Thunnus thynnus, Atomic absorption spectrometry, Chemometric classification*

Doğa ve Çiftlik Orkinoslarında (*Thunnus thynnus* L1758) Tespit Edilen Bazı Elementlerin Kemometrik Sınıflandırması

Özet

Orkinos *Thunnus thynnus* 1758 (BFT) Scombridae familyasına ait olup, Atlantik Okyanusu, Ortadoğu ve Karadeniz'in subtropik bölgelerinde yaşayan doğal türlerindendir. 30 adet doğadan yakalanan, 30 adet orkinos çiftiğinden alınan toplam 60 *T. thynnus* örneğindeki esansiyal elementlerin (Cu, Fe, Mg, Mn, Ni, Zn) ve esansiyal olmayan toksik elementin (Pb) konsantrasyonları alev atomik absorpsiyon spektrometresi kullanılarak tayin edilmiştir. Besleme orkinoslar, kafeslerden alınırken doğal olanları büyük balık ağlarıyla yakalanmışlardır. Orkinosların beslenme şekillerini ve farklı dokularının farklılandırılması, sınıflandırılması ve karakterize edilmesi için örnek tanımlama yöntemleri kullanılarak ön kemometrik çalışma yapılmıştır. Esas bileşen analizi (PCA), K en yakın komşular (KNN) gibi çok değişkenli kemometrik yöntemler, orkinosları türüne gore ve kimyasal bileşen temeline dayanarak sınıflandırmak için kullanılmıştır. Kararlı oldukları için metal konsantrasyonları sistemin sınıflandırılması için iyi bir aday olarak görülmektedir. Bu çalışma, PCA işlemlerinin doğada ve kafesde büyütülen orkinosların farklılıklarının ortaya konmasında ve sınıflandırılmasında yararlı bir yöntem olduğunu ortaya koymaktadır.

Anahtar sözcükler: *Eser elementler, Thunnus thynnus, Atomic absorpsiyon spektrometresi, Kemometrik sınıflandırma*

INTRODUCTION

The bluefin tuna, *Thunnus thynnus L*. 1758, is a long-lived and fast growing pelagic species with spawning migratory behaviour [1]. The bluefin tuna (BFT) migrate annually between the Black Sea and the Aegean and the Eastern Mediterranean. During the migratory session BFT feed intensively with different nutrients and various aquatic species. Commonly, BFT are captured by purse seine in these period and it leads to the overfishing. Thus, BFT should be listed as an endangered fish species in Atlantic Ocean, since 1992 [2].

İletişim (Correspondence)
+90 232 3114066
ozlem.sogut@ege.edu.tr

The high commercial value and increasing demand make the bluefin tuna a suitable species for farming. Since the 2002s, a cage fattening industry has developed in Antalya Bay and Ildir Bay, located in Cesme-Izmir, Turkey. The quality of farmed tunas arises from several factors to do with farming and environment [2].

Trace elements are required for normal life processes, and all animals including fish need these inorganic elements. Fish may derive these elements from diet and also from ambient water. Increased levels of trace elements are indicative of bioaccumulation of heavy metal toxins, mainly in liver tissues. Many fish species are threatened by such toxins and they are used to investigate the impact of environmental pollution [3-5].

Copper (Cu) is an essential trace element for all organisms, including fish; it plays an important role in organism's metabolism and its concentration is well regulated. Cu is one of the most toxic metals to fish and has various effects, including increased liver enzyme activity, liver diseases [6]. Iron (Fe) is another essential but potentially toxic element for animal organisms that is stored via a number of ways, including hemoglobin and myoglobin in erythrocytes, other proteins including ferritin and hemosiderin in serum and body tissues especially the liver. Iron is used for enzymatic reactions. Fe deficiency may leads to anemia, hemolytic problems and liver diseases [6]. Magnesium (Mg) is essential like other macro elements in animal organisms. Mg serves as a cofactor of phosphohydrolases and phosphotransferases [7]. Mg depletion (Hypomagnesemia) leads to weakness, tetany, erratic swimming and central nervous system disease, however, hypermagnesemia potential cardiac effects, renal disease. Manganese (Mn) is essential like other trace elements in animal organisms. Mn is necessary for the normal functioning of brain and proper lipid and carbohydrate metabolism. The absence of Mn in fish meal diet significantly influenced the mineral composition of common carp gonads [6]. Nickel (Ni) can do no harm to an organism unless the organism absorbs the divalent nickel ion into its body or the nickel ion is bound strongly enough to a membrane (fish gills) so that the membrane cannot function properly. Ni deficiency in the liver is decreased concentrations of Zn in liver [8,9]. Zinc (Zn) is essential element for aquatic life, for example, it occurs in the enzyme carbonic anhydrase, which catalyses the formation of carbonic acid from carbon dioxide in the blood. At high levels, Zn exert a direct toxic action [6]. Although lead (Pb) has a high profile in human toxicology, it is of much lesser importance for aquatic life. The effects of Pb on central nervous system cause anemia, renal failure and increase of liver enzymes. Large predators, such as bluefin tuna, are at the top of aquatic food chains and hence they can accumulate large amounts of metals. In addition those pelagic organism, particularly tuna, are high-performance fish with very high metabolic rates and consequently the rate of food intake is elevated, a property which contribution notably to the accumulation of pollutants [10].

The aim of this study was to develop a method to confirm the wild BFT as farmed BFT. In this work, we present the results of application of pattern recognition methods to key metals data in BFT to differentiate between wild and farmed ones. The basis of classification procedure was the metal accumulation of different parts of BFT samples. Metal concentration seems to be good candidates for classification system, as they are stable; although tunas are acknowledged to be top-level predators able to concentrate large quantities of metals and represent a dietary source for humans, to our knowledge only few papers have dealt with metal determination in the Mediterranean [2,9,11-14] and there is no classification paper on tunas.

MATERIAL and METHODS

Sampling

Wild BFT were obtained using purse seiners from the bay of Antalya, Levantene Sea, during spring and early summer in 2008. Farmed BFT samples were taken from the cages of fish farm in Ildir Bay, Izmir, at the same time. The cages were conical, having 50 m surface diameter, 30 m depth and 30 m bottom diameter. The depth of sea was between 40 and 60 m. Physical parameters of sampling areas, like dissolved oxygen, temperature and salinity were measured by Oxyguard Handy Gamma (OxyGuard Int. A/S, Denmark). Fork length, total weight and gender of each BFT were determined. Wild samples were taken onto the deck approximately 15 min after capture. BFT were slaughtered and gutted and then liver samples were obtained and stored in CO_2 ice. Samples were transferred to the laboratory and washed with distilled water, dried in filter paper, homogenized, packed in polyethylene bags and stored at -80°C prior to analysis. Farmed samples were taken from the cages, transferred to the laboratory and prepared for analysis using the same method.

Digestion Procedures

Liver, heart and meat tissues (head and tail meat) with a wet weight of 0.5 g were dried for 48 h at 110°C. Samples were digested with a mixed solution of perchloric 70% and nitric 65% acids (3:7, v/v) and slowly heated 80°C until complete digestion. De-ionized water, from Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all aqueous solutions. The perchloric acid, nitric acid and standard solutions (1.000 mg/L) were supplied by Merck (Darmstadt, Germany).

Apparatus

A flame atomic absorption spectrophotometer (Varian SpectrAA-10 Plus model) was used for the analysis. The zinc, iron, nickel, manganese, copper and lead hollow cathode lamps were used according to the manufacturer's recommendations.

Data Analysis

Each sample was considered as an assembly of 7 variables represented by the chemical data. These variables called 'features' formed a 'data vector' which represented a BFT sample. Data vector belonging to the same group (wild or farmed) were analysed. Pattern recognition tools used in this work as follows:

Autoscale

This is the most widely used scaling technique. The procedure standardizes a variable *m* according to:

$$Y_{mj} = \left(\frac{(X_{mj} - \overline{X}_m}{s_m} \right)$$

where Y_{mj} is the value *j* for the variable *m* after scaling, x_{mj} is the value *j* for the variable *m* before scaling. X_m is the mean of the variable and *sm* is the standard deviation of the variable. The result is a variable with zero mean and a unit standard deviation. Standardization is useful in areas such as quantitative structure- properly relationships where many different pieces of information are measured on very different scales [15].

Principal Component Analysis (PCA)

This procedure was used mainly to achieve a reduction of dimensionality, i.e. to fit a *j*-dimentional subspace to the original p-variate (*p*> >*j*) space of the objects and pit allows a primary evaluation between-category similarity [14].

K Nearest Neighbour (KNN)

This method, based on the distance between objects in the p-space as its criterion, is used to classify an object in the category which contributes the greatest number of the K- nearest known objects. It is a non parametric method in as much as it does not formulate a hypothesis on the distribution of the variables used. Only the closest K objects are used to make any given classification. The importance of a given feature in taking the decisions is proportional to its contribution to the distance calculation. The inverse square of Euclidean distance was used in this work [14,15]. Euclidean distance is the distance between two samples *k* and *l* is defined by:

$$Y_{mj} = \left(\frac{(X_{mj} - \overline{X}_m}{s_m} \right)$$

where there are *j* measurements and x_{ij} is the j_{th} measurement on sample *i*.

Pattern recognition analysis were performed by means of statistical software packages MATLAB (Matlab 7.0)

RESULTS

Ten water samples were analyzed for each site for each season, to check the water quality. Mean±SE values of dissolved oxygen, temperature and salinity parameters for Ildir Bay were 8.10±0.7 mgL^{-1}, 15.3±1.5°C, 36.5±0.05 gL^{-1} in winter and 7.90±0.5 mgL^{-1}, 15.7±2.2°C, 36.2±0.07 gL^{-1} in spring. Values for Antalya Bay were 7.85±0.2 mg L^{-1}, 16.5±1.2°C, and 37.2±0.06 gL^{-1} in winter and 7.60±0.3 mgL^{-1}, 17.0±1.7°C, 37.5±0.09 g L^{-1}, respectively, in spring.

A total of 60 individuals of *T. thynnus* were collected: 30 captured in the wild and 30 sampled from a tuna farm. In both wild and farmed BFT, 15 individuals were female and 15 were male. The fork length and weight of the 15 wild BFT were 1524±1.7 mm and 1530±1.4 mm, compared with 56.5±3.6 kg and 53.8 kg±3.2 kg for the farmed fish. The fork length and weight of female wild, female farmed, male wild and male farmed were 1540±1.9 mm and 58.6 kg±3.9 kg, 1551±2.1 mm and 55.5 kg±3.8 kg, 1508±1.2 mm and 54.4±2.7 kg and 1509±1.2 mm and 52.1 kg±2.4 kg, respectively.

The results of the metals determined in wild and farmed and bluefin tuna samples are summarized in *Table 1* and *Table 2*. The accumulation of the elements in the parts of the BFT was evaluated. PCA was used to provide a partial visualisation of data in a reduced-dimension plot. Examining score plot of samples in the space defined by the two first principle components in *Fig. 1*. A natural

Table 1. *Concentration of some trace metals in different parts Northern Bluefin Tuna (mg/kg wet weight)*
Tablo 1. *Orkinosların farklı bölgelerindeki bazı eser element miktarları (mg/kg yaş ağırlık)*

PARTS OF BFT	Metal concentrations							
	Cu		Fe		Mg		Zn	
	Wild	Farmed	Wild	Farmed	Wild	Farmed	Wild	Farmed
Head	5.09±5.36	6.44±4.11	53.7±6.34	54.4±3.81	53.7±6.34	54.4±3.81	41.6±13.5	37.9±30.2
Tail	2.76± 3.18	5.90±1.16	49.2± 4.11	40.8±5.34	49.2± 4.11	40.8±5.34	49.0±7.05	33.8±22.6
Heart	6.04±5.14	5.5 ± 1.74	38.1± 4.72	39.1±4.62	38.1± 4.72	39.1±4.62	30.4±7.12	26.9±11.3
Liver	5.47±2.70	7.17±3.32	49.7± 11.7	43.3±6.25	49.7± 11.7	43.3±6.25	193.5±59.31	126.5±46.6

Mean±SE: Mean±Standart Error

Table 2. *Concentration of some trace metals in different parts Northern Bluefin Tuna (mg/kg wet weight)*
Tablo 2. *Orkinosların farklı bölgelerindeki bazı eser element miktarları (mg/kg yaş ağırlık)*

PARTS OF BFT	Metal concentrations					
	Mn		Ni		Pb*	
	Wild	Farmed	Wild	Farmed	Wild	Farmed
Head	1.12±0.43	0.59±0.74	0.85±0.36	1.01±0.27	0.62±0.49	0.56±0.26
Tail	0.96±0.35	1.39±0.41	0.65±0.38	1.23±0.29	0.36±0.34	0.68±0.30
Heart	1.23±0.62	1.25±0.42	0.86±0.49	1.29±0.68	0.43±0.35	0.41±0.36
Liver	1.29± 0.30	1.37±0.48	0.71±0.29	1.08±0.69	0.32±2.23	0.49±0.23

*Mean±SE: Mean±Standart Error * Pb is an non-essential toxic element*

separation between wild and farmed BFT was found. It is not only scores, however, that are of interest, but also sometimes the loadings. Exactly the same principles apply in that value of loadings at one PC can be plotted against that at the other PC. The results show in *Fig. 2*. Some loadings are in the middle of the loadings plots such as Cu, Fe, Mn and Zn.

The KNN is a grouping method. Using nearest neighbour linkage and correlation coefficients for similarities, the dendrogram is presented in *Fig. 3*. It can be seen that accumulation of trace elements in both wild and farmed liver are very different from the others. The other objects appear to form two groups; wild and farmed.

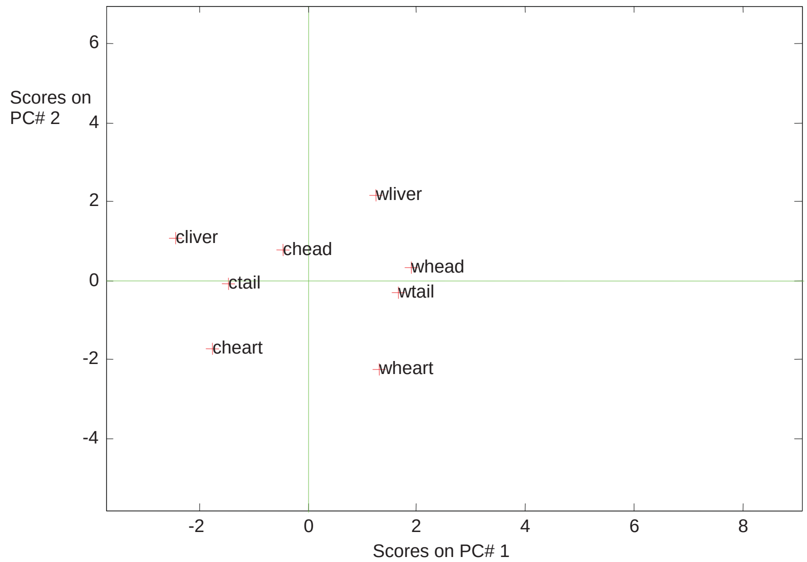

Fig 1. Comparison of score plots according to the trace element levels of BFT

Şekil 1. Orkinoslardaki eser element düzeylerine göre skor noktalarının karşılaştırılması

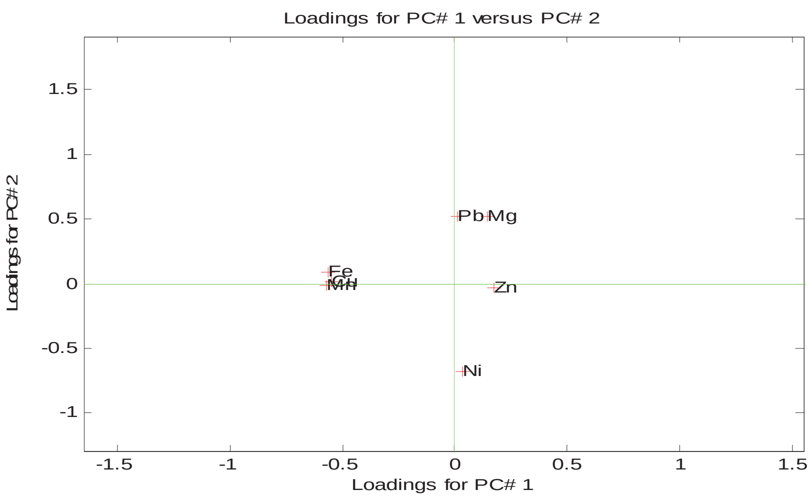

Fig 2. Comparison of loadings plots according to the trace element levels of BFT.

Şekil 2. Orkinoslardaki eser element düzeylerine göre loading noktalarının karşılaştırılması

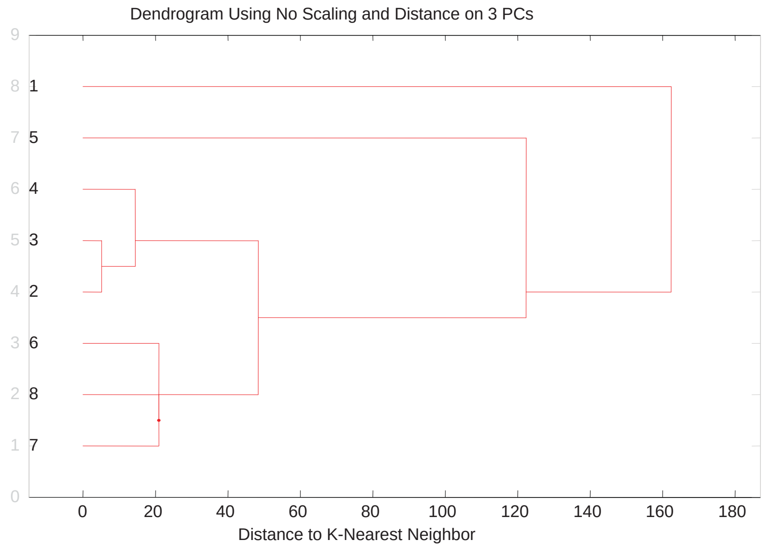

Fig 3. Dendrogram for trace elements in BFT by using PCA and KKN **1:** Wild liver, **2:** Wild head, **3:** Wild tail, **4:** Wild heart, **5:** Farmed liver, **6:** Farmed head, **7:** Farmed tail, **8:** Farmed heart

Şekil 3. KKN ve PCA kullanılarak orkinoslara eser elementler için dendogram **1:** Doğa karaciğer, **2:** Doğa baş, **3:** Doğa kuyruk, **4:** Doğa kalp, **5:** Kafes karaciğer, **6:** Kafes baş, **7:** Kafes kuyruk, **8:** Kafes kalp

DISCUSSION

Trace elements are essential in all living organisms and many are micronutrients in the diet of fish and mammals. Although the specific dietary requirements for trace elements (copper, iron, zinc, manganese, nickel, etc.) are largely unknown in fish, minor changes in the concentration of these elements have been shown to cause depressed health and growth states. Elevated metal concentrations in the marine environment are widespread and a potential health hazard to fish and mammals [17].

The relationship between concentrations of trace elements in fish tissues, their harmful effects and greater information on the levels of these substances in fish and their predators are important because marine species form a significant component of many human diets. In this study, the accumulation of the some elements in different parts of both wild and farmed the BFT was evaluated. The levels obtained were similar to those found by other authors [2,9,11-14,18].

The search for natural grouping in the samples is one of the main ways to study structure of the data. Principal component analysis (PCA) was used to provide a partial visualisation of data in a reduced-dimension plot. The principal components or eigenvectors are orthogonal and they are linear combination of the original variables [19]. A natural separation between wild and farmed BFT was found by PCA *(Fig. 1)*. In this factor space, wild BFT formed a group as like farmed ones. The standardisation puts all the variables on approximately the same scale. Hence the variables of low intensity assume equal significance to those of high intensity. If two scores plots provide comparable information, the less number of analyses are just as good as the full sets of tests. This can be of significant economical benefit.

The loadings plots give some information about the analysis *(Fig. 2)*. Some loadings are in the middle of the loadings plots such as Cu, Fe, Mn and Zn. These behave atypically and are probably not useful indicators of separation. Cu, Fe, Mn takes place as a group in *Fig. 2*. All of these are essential element for fish nutrition for nerve system and some enzymatic reactions. If excess amount of the elements are ingested and assimilated, toxicity may develop [6]. Zn and the group of Cu, Fe, Mn are almost diametrically opposed, suggesting that they measure opposite properties. Most loadings are on an approximate circle. This is because standardisation is used, and suggested that we are probably correct in keeping only two components. Nickel shows discrimination but closer to Zn. It is suggested that the reason should be the relation between Ni level and Zn in liver. Ni deficiency in the liver is decreased concentrations of Zn in liver.

The similarity of the new group from all other groups is given by the highest similarity of either of the original object to each other objects. KNN is a grouping method. The results of hierarchical clustering is presented in the form of dendrogram. The objects are organised in a row, according to their similarities. It can be seen that accumulation of trace elements in both wild and farmed liver are very different from the others in dendrogram obtained by using nearest neighbour linkage and correlation coefficients for *(Fig. 3)*. The other objects appear to form two groups; wild and farmed.

This study clearly shows that PCA procedures appear useful tools for differentiation and classification of wild and farmed BFT using the profile of trace elements. These findings might be a special relevance for farming in order to assure an adequate content nutrient of adapted.

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