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COMPONENT ANALYSIS OF THE DIFFERENT FISH SAMPLES CONTAINING HEAVY METALS IN ISTANBUL BOSPORUS

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ABSTRACT

Fish and fish products are important sources in nutrition, especially for kids and youths. Turkey has the geographical advantage of being surrounded on three sides by the sea. However, the usage of seas unconsciously and fishing illegally cause pollution, which threatens human health, and decrease fish rates.

In this study, distinguishable heavy metals are found (Pb, Cu, Ni, Al, Zn, etc.) that may affect human beings permanently in a negative way. The fish samples are analyzed, and significant heavy metal levels are displayed. The levels of heavy metals are introduced by using a mathematical approach and a statistical modeling. *Original* and *new* data are obtained from fishes related to levels of heavy metals. Data are analyzed by means of correlation-regression that is a linear model and a factor analysis method. Besides, regression equations and factor loadings are exhibited.

KEYWORDS:
Heavy metals, Factor analysis, Statistical model

INTRODUCTION

Determination of the toxic heavy metal levels in seafood is important for community health. Therefore, much more researches have focused on the effects of heavy metals on humans. Yang et al. investigated the concentrations of heavy metals (Cr, Cd, Hg, Cu, Zn, Pb and As) in water, sediment, and fish from the middle and lower levels of the Yangtze River, China [1].

The health risk analysis of each heavy metal in fish tissues indicates the safe levels for the general population and fishermen, but there is a possible risk in total target hazard quotients [1]. Heavy metal concentrations are investigated in Gaza Strip. It is studied to get information about heavy metal concentrations in the muscles of six commercial fish species available in Gaza Strip markets and the possible risk associated with their consumption is evaluated [2]. The amount of Cd, Cu, Cr, Hg, Ni and Zn in the muscles, livers and gills of whitefish, perch, pike, brown trout, burbot and vendace, which are taken from the lake in the vicinity of mining activity and several metallurgic smelters between Norway and Russia, is searched [3]. The levels and bioaccumulation of the organochlorine pesticides (OCPs) and heavy metals in muscles and livers of three fish species, with two trophic levels, from Lake Awassa, Ethiopia, are studied [4]. In Turkey a comprehensive study has been planned in 2010 and carried out to determine the radioactivity levels and heavy metal concentrations in the most common four fish species samples collected from eight stations in the Black Sea Region, which is affected by Chernobyl in 1986 [5]. Heavy metal (Cd, Cr, Cu, Hg, Pb, Zn) concentrations determined in the muscle tissues of the seven fish species (silver carp Hypophthalmichthys molitrix, grass carp Ctenopharyngodon idellus, crucian carp Carassius auratus, carp Cyprinus carpio, Coreius heterodom, catfish Silurus asotus, and yellow-head catfish Pelteobagrus fulvidraco) in Yangtze River are measured [6]. The heavy metal pollution in marine life has been taken into consideration as a serious environmental issue [7,8]. The pollutants are potentially accumulated in sediments and livings in seas and subsequently they are transferred to people through nutrition [9]. Metals, playing an important role in biological system, such as iron, copper, zinc and manganese, are essential metals, whereas mercury, lead and cadmium, which are toxic even in small amounts, are non-essential metals [10,11]. A formula is obtained for the quantification of benefit-risk ratio (hazard quotient) for the intake of a product containing essential polyunsaturated fatty acids e.g, heavy metals [12]. Monitoring fishes and shells is important for water ecosystem because people may get affected by nutrition [13]. Eventually, various researches have been carried out on metal accumulation in different fish species [14-18]. Fortunately the situation is not inextricable for now [19].

In the seas of Turkey, the investigation of heavy metals assessment (for two commercial fish species) is extensive [20]. The studies show that the high concentrations of metals in fish samples may be related to industry [20]. In addition, it is found that heavy metal levels are low in fish species sampled from relatively unpolluted areas [20,21]. The statistical comparison revealed that the metal concentrations are significantly different in each tissue of different fish species. There is a negative relationship between fish sizes and metal levels in most cases. The data show that the positive relationship is only between zinc and lead levels in the gill of Mugil cephalus and size. The relationship between the size of fish and metal concentration in the marine life should also be monitored occasionally in the field to understand better the effects of metals on fish growing and the current situation of population dynamics [21].

Heavy metal concentrations of the fishes in Beymelek Lagoon (Antalya/Turkey) are lower than those from other contaminated Mediterranean regions of Turkey. This research shows that the heavy metal concentrations in muscles of the observed species are also lower than the maximum levels set by law [22].

The results may be considered as a bioindicator of the contamination by estimating the bioavailability of metals in marine biota. Moreover, these results can also be used to test the chemical levels of the sea food and the possible risk associated with their consumption can be evaluated [23]. The method that is used for the new and original data is remarkable [24,25].

The goal of this study is to analyze the heavy metals statistically with the data that is implemented by data analysis, correlation-regression, general linear model, dendogram, factor loadings and regression equations.

MATERIALS AND METHODS

Sampling. Barb fish species used in this study (Red Mullet- Mullus barbatus), horse mackerel (Mackerel- Trachurus spp Horse), bluefish (Blue fish- Pomatomus saltatriks), sea bass (Seabass - Dicentrarchus labrax), Bonito (Bonito-Sarda sarda), Zargan (needlefish -Belo to vulgaris), Swallow (Red Gurnard- Triglidae), Haddock (Whiting fish-Merlangius merlangus), anchovy (Anchovy-Engraulis encrasicolus), mussels (Mussel-Mytilus galloprovincialis), Blue Fish (Young Blue fish-pomatomus saltatrix) Shrimp (Shrimp-Lysmat to rathbuna), the sardine (Sardina-Sardina pilchardus) are chosen. This analysis deals with the fish species in the Sea of Marmara, which are the most commonly consumed and economic in Februaries and Marches. Moreover, those ones affected by the heavy metals are joined in national and international trade and other industrial activities.

Analytical procedures. The fish samples in polyethylene containers together with ice are brought to the laboratory on the same day [20]. Firstly, the samples are washed with tapwater and then distilled water [27]. The liver and muscle samples taken from the big (sea bass, bonito, bluefish and Zargan), and small fish species are cut into small pieces with a plastic knife and dried in a 90 ° C-oven down to fixed weight and the ground has been homogenized [15,26].

0.25 grams of the dried tissue samples are mixed in PTFE containers (PTFE vessel) and by adding HNO3-H2O2 (5: 2, v / v) mixture it is put into the microwave for solubilization process [28,29]. The samples taken from microwave are completed to 50 ml with ultrapure water. The levels of heavy metal accumulation in examined tissues are determined by optical emission spectrometry with the 7000 DV ICP-OES model of brand Perkin Elmer Samples are passed through a 0.45 mm membrane filter before analysis.

Li, Ba, V, Cr, Fe, Ni, Cu, Zn, Al, Pb, Mn, Cd, Co, Sr, Na, K, Mg, Ca, P in the analyzed samples has been examined and the results are given in terms of mg / kg.

Element analysis. 0.2-0.5 gram of homogenously mixed samples is put into teflon containers in their dry forms. 9 ml pure HNO_3, 3 ml HCl and 2 ml HF are added for soil while this is 8 ml pure HNO_3 for plants (and diluted HNO_3 for tissues). The teflon containers are capped and placed in a microwave oven. The heat is gradually increased up to 185 °C and the container was kept at this heat for 20 minutes. Then, teflon containers are taken out and contents are poured to 50 ml HDPE volumetric flasks where they were complemented to 50 ml volume by addition of ultra-pure water. After those processes the conditions are convenient for heavy metal analysis at ICP-OES. The elemental heavy metal analysis of the collected soil samples has been carried out by Perkinelmer for the brand model (Optima 7000 DV) ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer).

In ICP, the sample collected through auto sampler is transferred to the nebulizer and therein it is mixed with argon gas to form aerosol. After that, it becomes plasm state at a temperature about 600-700°K. The 'Torch' where the plasm is constituted is composed of 2 nested quartz canals. Aragon is injected via two outside canals while the sample and aragon are injected together through small inside canals. The sample, which is sprayed with aragon through the injector, is brought into a form of plasm after being induced with an RF (Radio

Frequency) signal applied to metal coil on the side of the area. Emissions from the hot spot in the middle of the plasm are used for the analysis.

Five standards and a blank are prepared in order to constitute the calibration curve. The concentration of the standards is as follows: STD 1 10 ppb; STD 2 25 ppb; STD 3 50 ppb; STD 4 100 ppb; STD 5 250 ppb.

In order to operate the device, its software program is turned on. The obtained values are used via a special method. Recommended reading (plasma view) for low concentrations (ppb levels) is axial. This option is also entered into the method. Sample information is created to state the locations of the samples in auto sampler. Before the calibration process, the standard of the highest concentration is viewed as a sample and peak correction is made accordingly. After the blank and standards are viewed, the calibration curve is drawn. Correlation coefficient is expected to have three or four 9's for the sensitivity of the calibration. Samples, whose flow is 1.5 ml/minute, are placed in the auto sampler. The device begins to read the sample in 45 seconds after the sample flow. It makes 3 readings for each sample and gives the result, as the average of them in terms of μg/L. Plasm speed is 17L/m. Nebulizer speed is 0.55 L/m. The washing process is carried out with 45-secondperiods.

Domestic, industrial, agricultural and chemical wastes threaten human health as well as the marine life. Toxic materials and heavy metals in fish species cause irreversible damages in neural system, muscle functions, respiration system, circulatory system, immune system and affect negatively the growth and many other functions of the organisms. The heavy metals accumulate in liver, kidney and spleen intensively. Due to the negative effects of the heavy metals as indicated above, the rates of them need to be investigated and analyzed statistically.

In this analysis that is based on environmental pollution, a descriptive analysis of variables, correlation matrix and dendogram of hierarchical cluster are investigated. Multivariable analysis exhibits interaction among variables. The variables are introduced on two-main axis. Pb, Co, Zn, Ni, Cu, V, Cr and K scatter in ±x, Mg, Cd, Al, Ca and Na scatter in ±y.

One of the sources of heavy metal pollution is also automobiles. The soil absorbs those metals spread from automobiles. This fact needs to be analyzed rigorously. The analysis reveals the correlation of the metals. For example, Na and K alkali metals have the same property with each other; Al, Mg, Cr, Ni and Cu soil alkali metals as well.

The descriptive analysis values of heavy metals in fish samples are shown in Table1. Accordingly the most potential damaging heavy metals are Pb, Cu, Cd, Mn respectively.

TABLE 1
Descriptive analysis

	N	Minimum	Maximum	Mean	Std. Deviation
Ba	36	0	1,09	0,1984	0,32728
V	36	0	0,29	0,0982	0,08092
Cr	36	0	0,74	0,2948	0,21158
Ni	36	0	5,17	0,1489	0,86040
Cu	36	0,66	12,84	3,8156	3,63103
Pb	36	0,00	,36	0,0974	0,13535
Mn	36	0,17	3,73	1,5638	1,20537
Cd	36	0	0,03	0,0029	0,00892
Co	36	0	0,12	0,0084	0,02634
Sr	36	1,00	50,60	9,8627	14,16121
Fe	36	2,20	186,21	41,9825	50,55500
Zn	36	7,33	107,20	41,8176	30,25427
Al	36	0	104,49	16,7064	26,13252
Na	36	1601,24	14618,97	5339,0807	3757,38811
K	36	4605,68	19028,21	12259,1377	4134,39235
Mg	36	459,94	2676,61	1385,3217	567,25131
Ca	36	193,02	5298,34	1843,5403	1413,30051
P	36	4722,57	12445,02	8013,1191	1850,88630

Correlations. The instant correlation coefficients of the heavy metal variables in fish samples between each other are shown in Table2.

TABLE 2
Pearson's correlation matrix for the metal concentrations

	Ba	V	Cr	Ni	Cu	Pb	Mn	Cd	Co	Sr	Fe	Zn	Al	Na	K	Mg	Ca	P
Ba	1	,410*	,288	-,106	,408*	,030	,692**	,282	-,192	,531**	-,026	,481**	,558**	,259	-,383*	,020	,880**	,045
V	,410*	1	-,087	,111	,389*	,616**	,373*	,087	,029	,586**	-,082	,182	,549**	,531**	-,257	,123	,373*	-,202
Cr	,288	-,087	1	,293	,577**	-,273	,688**	,097	,016	,406*	,709**	,632**	,405*	,546**	,232	,694**	,513**	,670**
Ni	-,106	,111	,293	1	,099	-,128	,161	-,048	,152	-,029	,348*	,273	,068	,083	,115	,287	-,043	,241
Cu	,408*	,389*	,577**	,099	1	,194	,666**	-,058	,155	,838**	,578**	,544**	,720**	,762**	-,252	,538**	,485**	,133
Pb	,030	,616**	-,273	-,128	,194	1	-,043	-,227	-,236	,479**	-,275	-,217	,311	,536**	,221	,288	,022	-,014
Mn	,692**	,373*	,688**	,161	,666**	-,043	1	,300	,086	,539**	,623**	,865**	,609**	,561**	-,167	,505**	,818**	,398*
Cd	,282	,087	,097	-,048	-,058	-,227	,300	1	-,104	-,053	-,057	,088	,220	-,033	-,302	-,125	,340*	-,069
Co	-,192	,029	,016	,152	,155	-,236	,086	-,104	1	-,139	,456**	,319	-,141	-,119	-,357*	-,229	-,238	-,247
Sr	,531**	,586**	,406*	-,029	,838**	,479**	,539**	-,053	-,139	1	,164	,270	,840**	,870**	-,222	,478**	,600**	,017
Fe	-,026	-,082	,709**	,348*	,578**	-,275	,623**	-,057	,456**	,164	1	,802**	,149	,367*	,089	,592**	,183	,502**
Zn	,481**	,182	,632**	,273	,544**	-,217	,865**	,088	,319	,270	,802**	1	,259	,338*	-,085	,459**	,618**	,465**
Al	,558**	,549**	,405*	,068	,720**	,311	,609**	,220	-,141	,840**	,149	,259	1	,737**	-,314	,357*	,590**	-,039
Na	,259	,531**	,546**	,083	,762**	,536**	,561**	-,033	-,119	,870**	,367*	,338*	,737**	1	,136	,774**	,494**	,333*
K	-,383*	-,257	,232	,115	-,252	,221	-,167	-,302	-,357*	-,222	,089	-,085	-,314	,136	1	,569**	-,209	,779**
Mg	,020	,123	,694**	,287	,538**	,288	,505**	-,125	-,229	,478**	,592**	,459**	,357*	,774**	,569**	1	,331*	,791**
Ca	,880**	,373*	,513**	-,043	,485**	,022	,818**	,340*	-,238	,600**	,183	,618**	,590**	,494**	-,209	,331*	1	,280
P	,045	-,202	,670**	,241	,133	-,014	,398*	-,069	-,247	,017	,502**	,465**	-,039	,333*	,779**	,791**	,280	1
*. Correlation is significant at the 0.05 level (2-tailed).																		
**. Correlation is significant at the 0.01 level (2-tailed).																		

TABLE 3
KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0,606
Bartlett's Test of Sphericity	Approx. Chi-Square	931,843
	Df	153
	Sig.	0,000

KMO and Barlett's Test tables (Table3) indicate the suitability of the data for structure detection. The **Kaiser-Meyer-Olkin Measure of Sampling Adequacy** is a statistics that indicates the proportion of variance in our variables that might be caused by underlying factors. High values (close to 1,0) generally indicate that a *factor analysis* may be useful with our data. Therefore our value is greater than 0,50, the results of the *factor analysis* probably will be very useful.

Dendogram of hierarchical cluster analysis of heavy metal concentrations in fish of Istanbul's seas are shown in Figure1. Metal clusters of variables are expressed in the figure hierarchical structure between these variables. Accordingly, there are two main heaps. The first phrase is Na, P, K which are beneficial substances. When the variables are substituted into the data reduction method, three maximum eigenvalues of covariance matrix describe 71,225% of total change. The number of variables is 18.

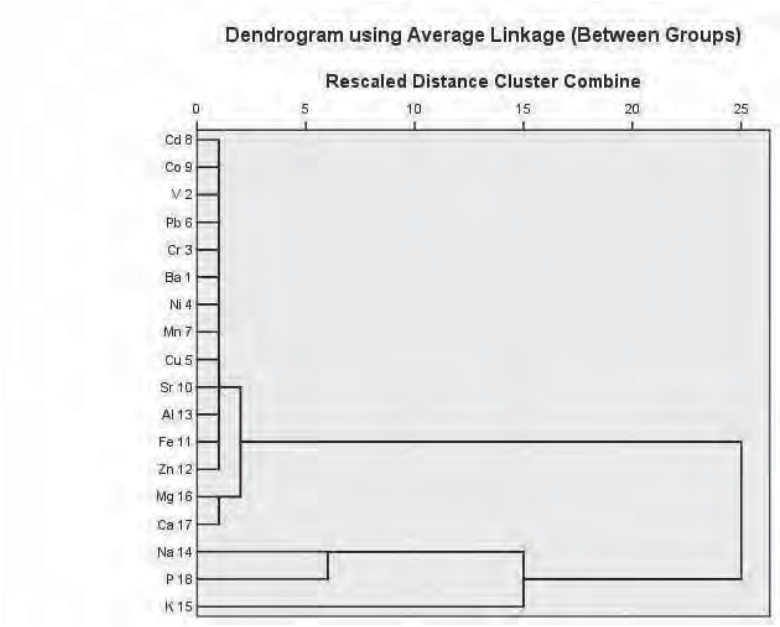

FIGURE 1
Dendogram for Variables

TABLE 4
Principal component analysis

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings	
	Total	% of Variance	Cumulative %	Total	% of Variance
1	6,085	38,004	38,039	6,847	38,039
2.	3,036	18,067	56,710	3,361	18,671
3.	2,061	14,052	71,225		

The first two-main axes explain 71% of total variance:

Y_1=0,133*Ba-0,056*V+0,884*Cr+0,398*Ni+0,535*Cu-0,113*Pb+0,676*Mn-0,066*Cd+ 0,048*Co+0,298*Sr+0,837*Fe+0,744*Zn+0,246*Al+0,557*Na+0,443*K+0,863*Mg+0,405 *Ca+0,841*P

Y_2=0,746*Ba+0,743*V+0,150*Cr-0,136*Ni+0,660*Cu+0,407*Pb+0,595*Mn+0,258*Cd- 0,095*Co+0,850*Sr-0,04*Fe+0,274*Zn+0,849*Al+0,625*Na-0,537*K+0,115*Mg+0,707 *Ca-0,257*P

TABLE 5
Total variance Rotated Component Matrix[a]

	Component	
	1	2
Ba	0,133	0,746
V	-0,056	0,743
Cr	0,884	0,150
Ni	0,398	-0,136
Cu	0,535	0,660
Pb	-0,113	0,407
Mn	0,676	0,595
Cd	-0,066	0,258
Co	0,048	-0,095
Sr	0,298	0,850
Fe	0,837	-0,04
Zn	0,744	0,274
Al	0,246	0,849
Na	0,557	0,625
K	0,443	-0,537
Mg	0,863	0,115
Ca	0,405	0,707
P	0,841	-0,257
Extraction Method: Principal Component Analysis.		
Rotation Method: Varimax with Kaiser Normalization.		
a. Rotation converged in 3 iterations.		

TABLE 6
Total variance Component Transformation Matrix

Component	1	2
1	0,736	0,677
2	0,677	-0,736
Extraction Method: Principal Component Analysis.		
Rotation Method: Varimax with Kaiser Normalization.		

When the variables are rotated with Varimax method, the projection of variables lie on the main axis. According to that, two groups can be obtained from the cluster analysis. The variables are scattered along two–main axes. The first one is V, Pb, Cd, Ba, Al, Sr, Ca, Cu, Na and the second one is Mn, Zn, Mg and Cr. Co, Ni, Fe, P and K elements scatter the negative axis for stability.

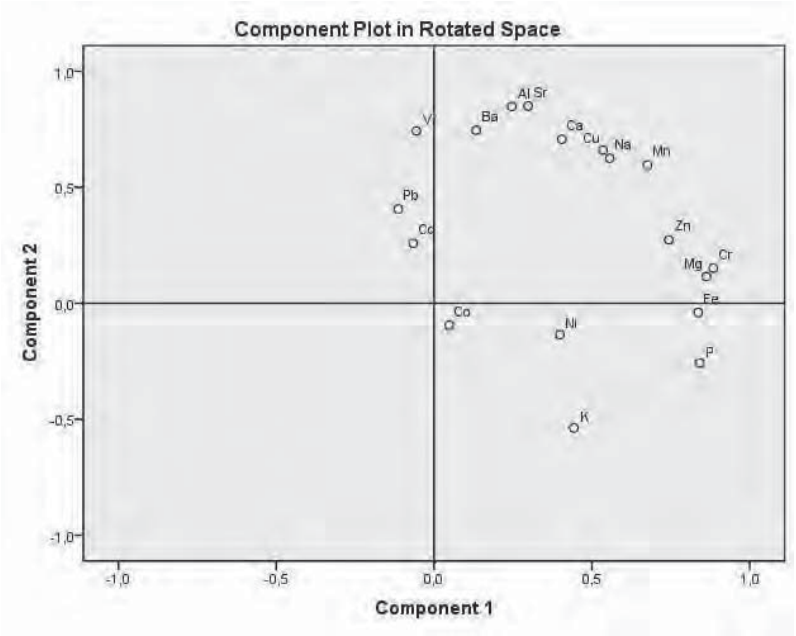

FIGURE 2
Factor Loadings for variables

CONCLUSION

This study is based on environmental pollution variables. It has yielded its statistical analysis from a descriptive analysis of variables, a correlation matrix and a dendogram of hierarchical cluster. The results of multivariable statistics are significant in that two main clusters can be observed.

The variables are introduced two-main axis in R^2 space and they are observed to lie ±x and ±y axis. Pb, Co, Zn, Ni, Cu, V, Cr and K scatter in ±x axis. Mg, Cd, Al, Ca and Na scatter in ±y. Those with the largest share of heavy metal in the fish could be identified in the factor loading of the two analyses. In the first axis the four significant elements are Cr, Mg, Fe and in the second axis they are V, Ba, Al, Sr respectively.

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