

## Determination Heavy Metal Levels of Some Feed Ingredients Produced in Tekirdag, Turkey

FISUN KOC\*, MAHMUT TUFAN†, E. KEMAL GURCAN and SERDAR GENC

*Department of Animal Science, Faculty of Agriculture,*

*Namik Kemal University, Tekirdag 59030, Turkey*

*Fax: (90)(282)2931480; Tel: (90)(282)2931442*

*E-mail: fkoc@nku.edu.tr*

Main objective of this study is to determine the pollution levels and province variations of polluting agents in some feed ingredients produced in Tekirdag and also to determine whether legal tolerance levels had been exceeded or not. In order to achieve this goal, cadmium, lead, arsenic, copper, zinc, iron analysis were performed during the fiscal year of 2007 from feed ingredients (wheat, sunflower, barley) in two provinces Tekirdag. Atomic absorption spectrophotometer (AAS) had been utilized for heavy metals. The data showed heavy metal level in feed ingredients for ruminants in Tekirdag were lower compared to current European regulations. The monitoring of these elements content is necessary as well, although not at the same level as, for instance for lead and cadmium.

**Key Words:** Heavy metal level, Wheat, Barley, Sunflower, Tekirdag.

### INTRODUCTION

The feed ingredients are strictly controlled with regard to two groups of substances (heavy metals, dioxin and PCBs, pesticide and mycotoxin residues and micro-organisms) and forbidden substances (recycled fats, non-filtered ruminant fats, unauthorized feed ingredients, processed animal protein, hormones and others, including some supplements, GMO and medications)<sup>1</sup>.

In Turkey, the European official regulations for maximum allowed undesirable substances and products used feed ingredients, are introduced under Act No. 2/2005<sup>2</sup>.

Heavy metals, being in the group of undesirable substances are a definite human health hazard because of their bioaccumulativity<sup>3</sup>. The extensive contamination of various foods and beverages with heavy metals as well as their constant and continuous use represent a serious risk to human health, the most dangerous being lead, cadmium and mercury<sup>4-8</sup>. Heavy metals are widely used in all fields of life (batteries, dyes, alloys, chemical compounds, pharmaceutical and cosmetic products)<sup>9-11</sup>. Thus suggesting that the risk of pollution is very high and therefore, the strengthened control along the entire food chain.

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†Agriculture and Rural Ministry Tekirdag Province Control Laboratory, Tekirdag, Turkey.

As far as the feed ingredients for ruminants are an integral part of the consumer's food chain, they need to be assessed as potential sources of heavy metal contamination.

Main objective of this study is to determine the pollution levels and province variations of polluting agents in some compound animal feeds produced in Tekirdag and also to determine whether legal tolerance levels have been exceeded or not.

## EXPERIMENTAL

**Trace elements analysis:** The elemental analysis of Cd, Pb, As, Cu, Zn and Fe was performed using Varian AA200 atomic absorption spectrophotometer equipped with a graphite furnace. A microwave system was used for the acid digestion of all of the samples. The feed samples were dried at 70 °C for 48 h in a forced stove. Dry sample (0.5 g) was added with 6.0 mL of HNO<sub>3</sub> (65 %), 1.0 mL of H<sub>2</sub>O<sub>2</sub> (30 %) and 0.5 mL of HF. The solutions were filtered and stored in PET bottles and deionized water added until the volume of the solution is 25 mL, while all of the others were stored in PET bottles with deionized water added until the volume of the solution reached to 50 mL. All of the samples were dried at 70 °C in a forced stove until of dry weight. Dry samples (0.3 g), finely crushed, was added with 6.0 mL of HNO<sub>3</sub> (65 %) and 1.0 mL of H<sub>2</sub>O<sub>2</sub> (30 %)<sup>12</sup>.

**Sampling sites:** All of the samples were collected during summer 2007 in 10 fields located in two regions of Tekirdag, Turkey (Fig. 1). The geographical features and the anthropogenic characteristics of the sampling sites using two aggregated indicators, which were defined follows: Index P is a cumulative index that takes into account the presence of different emission sources (high volume of traffic, industrial plants, dumps, waste incinerators, metropolitan areas) Q is a small-medium towns (intensive agricultural activity).



Fig. 1. Study area

**Statistical methods:** Descriptive statistics and regarding to kind of feed and analysis of variance according to the region were executed<sup>13</sup>. The similarities according to the region were visualized by hierarchical cluster analysis and shown as form of dendrograms<sup>14</sup>. All statistical analysis were done by Minitab software<sup>15</sup>.

## RESULTS AND DISCUSSION

The higher Pb and Cd levels in animals feed samples around different industrial areas and contaminated environment have been reported by several workers<sup>16,17</sup>. Emissions from the industries and contamination of the pastures with the effluents lead to higher concentrations of toxic pollutants in feedstuffs, soil and water that results in enhanced body burden of toxic heavy metals through continual ingestion of contaminated feedstuff and water<sup>18</sup>. The risk to the animals around industrial areas including lead mines and metal industries depends on proximity of the factory and the age of the animals.

Table-1 shows the toxic and trace elements level in feed ingredients. The permissible Pb content in feed ingredients according to current official regulations is 1.00 ppm. The very low levels of the toxic metal, especially Pb in the samples indicates that these feed components the safe limits for toxic elements specified by the standards (Table -1). Moreover, Cd and As content of all the samples lower than analytically detectable limit levels (0.001 ppm).

Trace elements such as Cu, Zn, Fe *etc.* are very much essential for normal growth, disease resistance, production and reproduction in farm animals. Many of the essential trace elements have clinical properties in common and there are interactions between these elements, thus, influencing the metabolism of each other. Absorption, utilization and excretion of many trace elements in animal body are greatly influenced by other trace elements or compounds in the diet and their levels in the body<sup>19</sup>. Heavy metals are among other factors that interfere with normal bio-availability of trace elements by competing with them in the process of absorption from the intestine<sup>20,21</sup>.

Zinc was detected in all feed samples. High levels of Zn have been found in sunflower samples. Copper was detected in all feed component but lower concentrations than Zn. However the Cu and Zn concentrations across the different groups was very similar.

**Correlation of toxic and trace elements between feed samples:** Table-2 also shows the correlation of Cu, Zn and Fe levels between feed samples. On the basis of results of analysis conducted on wheat samples correlation relations among the metals, positive correlations were detected between Zn and Cu ( $r = 0.30$ ) and Fe and Zn ( $r = 0.48$ ). Negative correlations were detected in case of Fe and Cu ( $r = -0.13$ ).

Barley samples correlation among the metals, positive correlations were detected between Zn and Cu ( $r = 0.59$ ), Fe and Cu ( $r = 0.23$ ) and Fe and Zn ( $r = 0.38$ ).

Sunflower samples correlation among the metals, positive correlations were detected between Zn and Pb ( $r = 0.11$ ) and Zn and Cu ( $r = 0.56$ ). Negative correlations were detected Cu and Pb ( $r = -0.11$ ), Fe and Pb ( $r = -0.24$ ), Fe and Cu ( $r = -0.03$ ) and Fe and Zn ( $r = -0.66^*$ ). Similarly, on the basis of correlations among the metals, it was thought that metals with moderately positive correlations have the same possible pollution sources.

TABLE-1  
RESULTS OF DESCRIPTIVE STATISTICAL ANALYSIS AND DATA  
REPORTED FOR HEAVY METAL LEVELS IN FEED INGREDIENT SAMPLES

Region	Heavy metal	Mean	SEM	VC %	Max (ppm)	Min (ppm)	Detection (%)
Wheat (n = 10)							
Q	Cd	ND	-	-	-	-	-
P	Cd	ND	-	-	-	-	-
Q	Pb	0.27	0.15		0.41	<0.001	40
P	Pb	0.17	0.14		0.28	<0.001	40
Q	As	ND	-	-	-	-	-
P	As	ND	-	-	-	-	-
Q	Cu	3.34 <sup>a</sup>	0.23	15.64	4.00	2.60	100
P	Cu	4.28 <sup>b</sup>	0.09	4.77	4.50	4.07	100
Q	Zn	24.19 <sup>a</sup>	0.80	7.44	26.40	21.58	100
P	Zn	25.02 <sup>a</sup>	1.75	15.68	28.85	18.68	100
Q	Fe	45.98 <sup>a</sup>	4.10	19.92	58.69	35.11	100
P	Fe	46.03 <sup>a</sup>	3.31	16.10	54.30	39.39	100
Barley (n = 10)							
Q	Cd	ND	-	-	-	-	-
P	Cd	ND	-	-	-	-	-
Q	Pb	0.004	0.019		0.098	<0.001	40
P	Pb	0.096	0.018		0.152	0.049	100
Q	As	ND	-	-	-	-	-
P	As	ND	-	-	-	-	-
Q	Cu	3.21 <sup>a</sup>	0.19	13.42	3.70	2.69	100
P	Cu	4.02 <sup>b</sup>	0.16	9.01	4.48	3.56	100
Q	Zn	19.47 <sup>a</sup>	2.20	25.24	26.51	13.40	100
P	Zn	23.91 <sup>a</sup>	2.73	25.57	28.09	13.41	100
Q	Fe	61.50 <sup>a</sup>	9.40	34.19	91.09	33.77	100
P	Fe	55.10 <sup>a</sup>	10.80	43.59	83.63	29.11	100
Sunflower (n = 10)							
Q	Cd	ND	-	-	-	-	-
P	Cd	ND	-	-	-	-	-
Q	Pb	0.63 <sup>a</sup>	0.24	85.26	1.28	<0.001	100
P	Pb	0.31 <sup>a</sup>	0.12	87.50	0.67	<0.001	20
Q	As	ND	-	-	-	-	-
P	As	ND	-	-	-	-	-
Q	Cu	2.71 <sup>b</sup>	0.15	12.31	3.14	2.21	100
P	Cu	3.19 <sup>a</sup>	0.14	10.46	3.76	2.92	100
Q	Zn	43.24 <sup>a</sup>	2.08	10.76	48.01	36.47	100
P	Zn	47.50 <sup>a</sup>	3.89	18.32	60.36	36.55	100
Q	Fe	85.31 <sup>a</sup>	4.97	13.04	93.76	70.54	100
P	Fe	85.89 <sup>a</sup>	7.78	20.25	109.13	62.76	100

ND = Non Detectable, <sup>a, b</sup> is statistical important in the same trace element.

TABLE-2  
CORRELATION COEFFICIENTS FOR WHEAT, BARLEY  
AND SUNFLOWER SAMPLES

Wheat	Cu	Zn	Barley	Cu	Zn	Sunflower	Pb	Cu	Zn
	Cu				Zn		0.59		Cu
Zn	0.30		Fe	0.23	0.38	Zn	0.11	0.56	
Fe	-0.13	0.48				Fe	-0.24	-0.03	-0.66*

Correlations is significant at the \* $p < 0.05$  level.

According to heavy metal contents of samples collected at 10 regions. Hierarchical cluster analysis dendrogram was drawn considering the distance coefficient. Q1-Q2, P3 and P4 region have similar characteristics. Moreover Q1, Q2, P2, P5, P1, Q5 and P3, P4, Q3, Q4 stations also show similar characteristics (Fig. 2.)

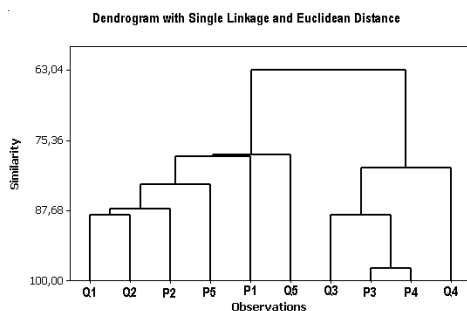


Fig. 2. Hierarchical cluster analysis dendrogram for each data matrix (wheat samples) in Tekirdag

Hierarchical cluster analysis dendrogram was drawn considering the distance coefficient Q1-P3, Q3-P1, Q4-Q5, Q2-P4 stations also show similar characteristics (Fig. 3).

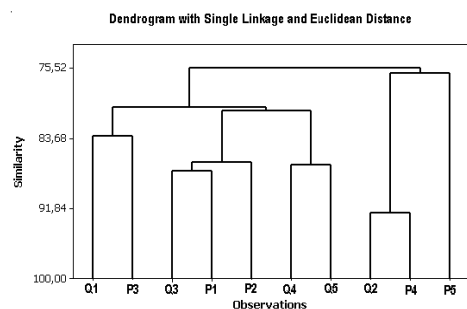


Fig. 3. Hierarchical cluster analysis dendrogram for each data matrix (barley samples) in Tekirdag

Hierarchical cluster analysis dendrogram was drawn considering the distance coefficient. P1-P3, Q2-Q4 region have similar characteristics. Moreover Q1, Q5, P1, P3, Q3 ve Q2, Q4, P2 stations also show similar characteristics (Fig. 4).

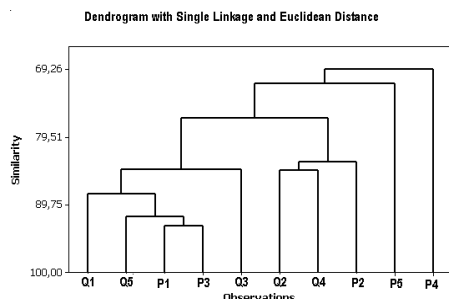


Fig. 4. Hierarchical cluster analysis dendrogram for each data matrix (sunflower samples) in Tekirdag

It was determined that the region with similar features have also similar element contents. In this respect, it is thought that is no different change along the region (Figs. 2-4).

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