

## EFFECT OF DISTANCE FROM THE ROADWAY ON HEAVY METAL CONTENT AND EGG QUALITY OF VILLAGE LAYING HEN'S EGG ALONG ROADSIDES OF TOKAT-TURHAL, TURKEY

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In this study, the heavy metal contents and egg quality characteristics of chicken eggs was studied. The eggs have been collected at distances of 0-100 (Distance A), 100-200 (Distance B) and 200-300 m (Distance C) away from the edge of Tokat-Turhal road. The distance from roadway had no significant effect on egg Cu and Mn contents ( $P>0.05$ ), although a significant effect on egg Cd and Pb content ( $P<0.05$ ) was observed. There were no significant difference in egg shape index, egg shell thickness, breakage strength, egg albumin pH, albumin index, Haugh units and yolk index according to distance from the roadway ( $P>0.05$ ). Eggs obtained from distance A and B had significantly higher weight than obtained from distance C ( $P<0.05$ ). Egg obtained from distance B had significantly lower egg shell colour scales than those of the other distances ( $P<0.01$ ). And, egg yolk colour was showed a significant variation among the distances away from the roadway, and the highest yolk colour value was detected in distance B ( $P<0.05$ ). Depending on obtained results, it can be concluded that the egg heavy metal concentrations exceeded background levels for hen eggs. Therefore, consumers should be aware of the possible health risks related by the use of these eggs which has obtained from side of road.

**Keywords:** Distance to roadway, laying hen, egg heavy metal content, egg quality, Turkey

### INTRODUCTION

There are sources of heavy metal pollution, like mining, agricultural and forestall waste disposal and fuel combustion which the main sources of heavy metals in the air traffic and long-range transport (Harrison *et al.*, 1981; Aslam *et al.*, 2011). Heavy metal pollutants play a potential role in assessing the quality of the roadside environment. Also, metals are persistent pollutants that can be biomagnified in the food chains, becoming increasingly dangerous to human and wildlife (Martinez-Lopez *et al.*, 2005). Metal accumulation varies with traffic density and distance from the highway (Grigalaviciene *et al.*, 2005). Analyzing pollutants in living organisms is more attractive and promising than analyzing pollutants of the abiotic environment, as living organisms provide precise information about the bioavailability of pollutants and the magnification and biotransference of pollutants (Phillips, 1977; Javed, 2012). Birds may be particularly suitable to investigate the presence and effects of contaminants in the environment. In addition to being conspicuous, ubiquitous and intensively studied, birds in many cases appear to be more sensitive to environmental contaminants than other vertebrates (Furness, 1993).

Also, heavy metal content in bird's egg reflects local

pollution levels (Burger, 2002), because hens live in close contact with the outside environment, they are exposed to contaminants (Furness and Greenwood, 1993; Chang *et al.*, 1989). In many investigations, analyses of roadside soils and vegetation have shown the concentration of heavy metal decreases with increasing distance from the roadway (Amusan *et al.*, 2003; Jaradat and Momani, 1999; Fakayode and Olu-Owolabi, 2003). Also, numerous studies have related the heavy metal contents of bird's eggs and internal organs come from environmental pollution (Burger, 2002; Hui, 2002; Mora, 2003; Burger *et al.*, 2004; Van Overmeire *et al.*, 2006; Sekeroglu *et al.*, 2007). Şekeroğlu and Akmaz (2009) reported that the distance from highway had no significant effect on egg iron, copper, zinc and lead contents, although a significant effect on egg manganese content was observed. The colour of eggshells may reveal much about environmental conditions and the health status of the female (Jagannath *et al.*, 2008). Eggshell quality of thin was among the first signs of detrimental effects of pollution (Ratcliffe, 1967). Thinner eggshells may reduce hatching success because eggs may break during incubation or eggs may dry out due to excessive evaporation (Drent and Woldendorp, 1989).

The objective of this study was to examine whether distance from the roadway had effect the heavy metals concentration

and egg quality in hen's egg.

## MATERIALS AND METHODS

Tokat province is located in the north middle east of Turkey, within  $39^{\circ} 52' - 40^{\circ} 55'$  north latitude and  $35^{\circ} 27' - 37^{\circ} 39'$  east longitude. Tokat-Turhal roads were chosen for the study. The road had average daily traffic density of 10336 vehicles (Anonymous, 2010). The main socio-economic activity along this road is farming. Egg samples were collected at distances of 0-100 (Distance A), 100-200 (Distance B) and 200-300 m (Distance C) away from the edge of Tokat-Turhal road (Fig.1).

In order to determine egg quality characteristics, 25 village hen eggs were collected from each distances (0-100 m, 100-200 m and 200-300 m) away from the edge of the roadway, the dry season (September), 2008. Egg samples have waited for 24 hours at room temperature. And then samples were taken for quality measurements. The following physical properties of egg samples were determined: length ( $L$ ) and width ( $W$ ). These were measured with a digital caliper to the nearest 0.01 mm.

The length and width of eggs was determined by egg shape measurer and their shape index (SI) was determined with following formula (Anderson *et al.*, 2004).

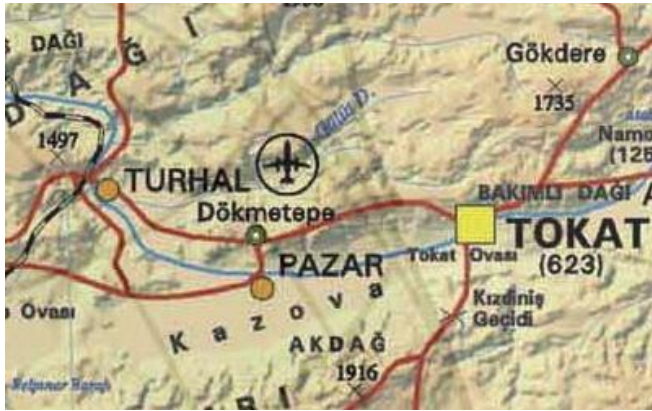


Figure 1. Map of Tokat-Turhal road where the samples were collected

$$SI = \left( \frac{W}{L} \right) \times 100$$

The egg weight (EW) was measured with an electronic balance to the nearest 0.001 g. Egg shell colour was measured using a Colorimeter 300B (Nippon Denshoku, Tokyo, Japan) (Flock *et al.*, 2001).

Shell thickness was measured according to Chawdhury (1987). Breaking strength (rupture force) was measured by the data acquisition system using Zwick/Roell (Instruction

Manual for Materials Testing Machines/BDO-FB 0.5 TS). The egg samples were placed on a moving platform with a loading position of 0.66 mm/s compression speed and pressed with a plate fixed on the load cell until the egg ruptured at X-axes (Şekeroğlu and Altuntaş, 2009).

Albumen and yolk height were measured using a sperometer. The yolk and albumen length were measured by electronic slide callipers. Yolk colour was measured using DSM Yolk Colour Fan (DSM, 2004). Individual Haugh Unit (HU) score was calculated using the egg weight and albumen height (Haugh, 1937). The Haugh Unit values were calculated for individual eggs using the following formula:

$$HU = 100 \log_{10}(H - 1.7 W^{0.37} + 7.57)$$

Where  $H$  is observed height of the albumen in mm and  $W$  is weight of egg in g.

Albumen index (AI) is related to the albumen height (AH), albumen length (AL) and albumen width (AW) and calculated with the following formula (Doyon *et al.*, 1986):

$$AI = \left( \frac{AH}{(AL + AW)/2} \right) \times 100$$

Yolk index (YI) is related to the yolk height (YH) and yolk width (YW) and calculated with the following formula (Doyon *et al.*, 1986):

$$YI = \left( \frac{YH}{YW} \right) \times 100$$

pH of the egg albumen was determined using a manually operated pH meter (Model 3310, 545 007/REV A/03-96).

In order to determine hen's egg heavy metal content, 10 egg samples were taken from each distance from the roadway. All reagents used in this study were analytical reagent grade unless stated otherwise. Double deionizer water ( $18.2 \text{ M}\Omega\text{cm}^{-1}$  resistivity) was used an aquamax™-Ultra water purification system (Young Lin Inst.) for all dilutions.  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  were of suprapur quality (E. Merck). All the plastic and glassware were cleaned by soaking in dilute nitric acid (1+9) and were rinsed with distilled water prior to use. The standard solutions of metal ions for calibration procedure were produced by diluting a stock solution of 1000 mg/L of the all the investigated element supplied by Sigma and Aldrich.

A Perkin Elmer A Analyst 700 model AAS with deuterium background corrector was used in this study. All measurements were carried out in an air/acetylene flame. The operating parameters for studying elements (Cu, Mg, Cd, Fe and Pb) were set as recommended by the manufacturer. Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi, maximum temperature  $300^{\circ}\text{C}$ ) was used. Teflon reaction vessels were used for all the digestion procedures. The reaction vessels were cleaned using 5 ml of concentrated nitric acid before each digestion. Egg samples were mixed up and approximately 2 g of samples were weighed in a Teflon vessel. The mixture of

HNO<sub>3</sub>: H<sub>2</sub>O<sub>2</sub> (6:2) was added into the vessel. Then, the microwave digestion programs were applied to the samples. Digestion conditions for microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, Vent: 8 min, respectively. After digestion completed, the samples were diluted to 15 ml with distilled water. The metal determinations were performed by a flame atomic absorption spectrometry. A blank digest was carried out in the same way for each digestion. All sample solutions were clear. The levels of the analytes in the blank solution were close to the detection limit of the method.

Egg mineral contents and egg quality parameter data were subjected to statistical analysis by the Generalized Linear Model Procedure of SPSS (SPSS 11.0). The data distribution was tested for normality by probit analysis and variance homogeneity by Bartlett test. Differences in the mean values were determined using Duncan multiple comparison test (Bek and Efe, 1989).

## RESULTS AND DISCUSSION

The results of egg quality obtained from the different distance from the roads were presented in Table 1. Eggs obtained from distance A and B had significantly higher weight than obtained from distance C ( $P<0.05$ ). Mean egg shape index were defined according to distance from the roadway and presented as %74.69 for distance A, %73.63 for distance B and %74.45 for distance C (Table 1). There was no significant difference in egg shape index according

to distance from the roadway ( $P<0.05$ ). Shell colour scales (L-a-b) changed from light to dark in the order of distance C (62.12), distance A (62.09) and distance B egg (51.26), respectively. Egg shell colour scales obtained from distance B had significantly lower egg shell colour scales than those in the other distances ( $P<0.01$ ). There was no significant difference between distances away from the roadway in terms of shell thickness and breaking strength ( $P<0.05$ ).

Albumen index was 8.09, 8.46 and 7.64 for distance A, B and C, respectively. No difference was found in the albumen index between distances away from the roadway ( $P<0.05$ ). The difference of Haugh unit of distances away from the roadway was not significant ( $P<0.05$ ). Also, distances away from the roadway had no effect on any of the egg albumen pH and yolk index ( $P<0.05$ ). But, egg yolk colour showed a significant variation among the distances away from the roadway, and the highest yolk colour value was detected in distance B ( $P<0.01$ ).

Effect of distances away from the roadway on hen's egg heavy metal content is shown in Table 2 and Fig 2. Egg Cu mineral contents were 1.28, 1.17 and 1.29 µg/g for distance A, B and C, respectively. There is no significant difference in egg Cu contents between distances away from the roadway ( $P<0.05$ ). The highest Mn concentration was obtained in the distance C eggs, while the lowest Mn concentration in the distance A eggs and average concentrations were determined in the distance B eggs ( $P<0.05$ ). But, in terms of Duncan multiple comparison test, there was significant difference in egg Mn contents

**Table 1. Hen's egg quality characteristics in relation to increasing distance from the roadway**

Egg quality characteristics	Distance, m			SEM	P
	Distance A	Distance B	Distance C		
Egg weight, g	58.08 <sup>b</sup>	57.48 <sup>b</sup>	53.65 <sup>a</sup>	0.76	<0.03
Shell colour scale (L-a-b)	62.09 <sup>b</sup>	51.26 <sup>a</sup>	62.12 <sup>b</sup>	1.58	<0.007
Shape index, %	74.69	73.63	74.55	0.33	0.45
Egg shell thickness, mm	0.356	0.349	0.346	0.003	0.41
Breakage strength, N	40.57	37.06	37.90	0.89	0.28
Egg albumin pH	9.05	8.97	8.4	0.04	0.70
Albumin index, %	8.09	8.46	7.64	0.27	0.54
Haugh units	82.13	82.06	80.39	1.11	0.79
Yolk index, %	39.46	41.63	40.64	0.50	0.29
Yolk color, RCS	11.45 <sup>a</sup>	12.47 <sup>b</sup>	11.88 <sup>ab</sup>	0.13	<0.01

Row means with different superscripts differ significantly at  $P<0.05$

SEM= Standard error of mean, P=Probability, RCS=Yolk Colour Fan

**Table 2. Hen's egg heavy metal content in relation to increasing distance from the roadway, µg/g**

Heavy metal	Distance, m			SEM	P
	Distance A	Distance B	Distance C		
Cu	1.28	1.17	1.29	0.04	0.48
Mn	0.36 <sup>a</sup>	0.45 <sup>ab</sup>	0.62 <sup>b</sup>	0.05	0.09
Cd	0.109 <sup>a</sup>	0.825 <sup>b</sup>	0.636 <sup>ab</sup>	0.13	<0.04
Pb	19.73 <sup>a</sup>	56.75 <sup>b</sup>	36.91 <sup>ab</sup>	5.25	<0.02

Row means with different superscripts differ significantly at  $P<0.05$ ; SEM= Standard error of mean, P=Probability

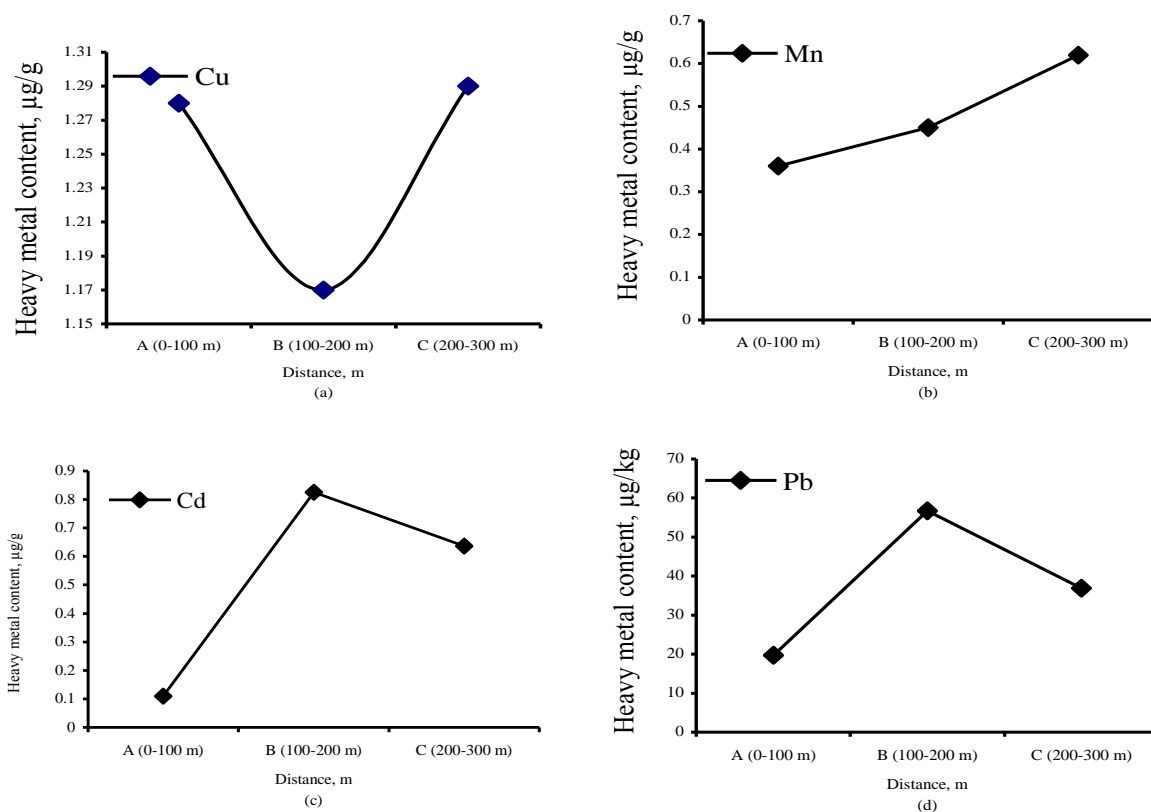
according to distances away from the roadway ( $P < 0.05$ ). Cd and Pb contents of eggs were found important statistically for the distances away from the roadway ( $P < 0.05$ ).

According to Schwaegelé (2001), egg shell colour related with hen health, genetic structure and hen ages. The colours of eggshells may reveal much about environmental conditions and the health status of the female (Jagannath *et al.*, 2008). Sekeroglu *et al.* (2007), put forward there were no important relations among egg shell colour properties and egg heavy mineral concentrations. In present study, egg heavy metal (Cd and Pb) concentration was significant in distance B higher than the others. In addition, egg shell color of distance B was significantly the darkest. The results of this work are in agreement with Sekeroglu *et al.* (2007).

While Ratcliffe (1967) reported that thinning of eggshells was among the first signs of detrimental effects of pollution, but in this study egg shell thickness was not statistically different between distances. In many investigations, analyses of roadside soils and vegetation have shown the concentration of heavy metal decreases with increasing distance from the roadway (Jaradat and Momani, 1999; Amusan *et al.*, 2003; Fakayode and Olu-Owolabi, 2003). By contrast, Şekeroğlu and Akmaz (2009) reported that to the

distance from highway had no significant effect on egg iron, copper, zinc and lead contents, although a significant effect on egg manganese content was observed. Also, they put forward that there were no important relations among distances away from the roadway and egg heavy mineral concentrations. But, in this study, the distance from highway had significant effect on egg Mn, Cd and Pb contents, except egg Mn content. Egg heavy metal concentration (Cu, Mn, Cd and Pb) recorded in this study was higher than concentrations found in hens' eggs of some researchers (Yang *et al.*, 1994; Doganoc, 1996; Dey and Dwivedi, 2000; Khan and Naeem, 2006; Van Overmeire *et al.*, 2006). In this study, the egg samples had Cu, Mn, Cd and Pb concentrations that exceeded 0.49 mg/kg, 0.28 mg/kg, 0.005 mg/kg and 0.020 mg/kg, respectively and these concentrations are normal background levels for egg heavy metal concentrations (Dey and Dwivedi, 2000).

**Conclusion:** The results obtained in this study showed that egg heavy metal (Cd and Pb) concentration was significantly higher in distance B than distance A and C. In addition, egg shell color and egg yolk colour of distance B was significantly the darkest. The egg heavy metal



**Figure 2.** Egg Cu contents (a), Mn contents (b), Cd contents (c) and Pb contents (d) according to distances away from the roadway

concentrations exceeded background levels for hen eggs. Therefore, consumers of hen's eggs obtained from side of road should be aware of the possible health risks connected by the use of these eggs.

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