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Possible heavy metal residues in poultry and their products that are bred around cement industry

Possíveis resíduos de metais pesados na carne de aves domésticas e seus produtos que são criados em torno da indústria de cimento

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Abstract Industry is an efficient tool for industrial development that is present in human lives and that mobilizes local and regional economies. When inspected from this aspect, industrial regions provide economic and social benefits for the societies. However on the other hand, they leave harmful effects to the environment and they may cause health and safety threats for communities. Mismanaged industrial regions may cause air and water pollution, noise problems and industrial accidents. One of the main purposes of this investigation is to determine the heavy metal (Pb, Cd, Cu, Cr, Co, Mo and Ni) levels in the liver, thigh and chest tissues of the chickens bred around the cement factory in a residential area. It is determined that the heavy metal levels in the livers of poultries bred in these areas are quite high (P<0,01) and also in the heavy metal analyses performed, the values obtained from the samples taken from the egg, thigh and chest tissues of the poultries are specified as high (P<0,01).

Keywords: ICP-AES (Atomic Emission Spectrophotometry), Heavy Metal, Liver, Poultry, Blood

Resumo A indústria é uma ferramenta eficiente para o desenvolvimento industrial, que está presente em vidas humanas e que mobiliza a economia local e regional. Quando inspecionados a partir deste aspecto, as regiões industriais proporcionam benefícios econômicos e sociais para as sociedades. No entanto, por outro lado, elas deixam os efeitos nocivos no meio ambiente e podem causar ameaças de segurança e saúde para as comunidades. Regiões industriais mal administradas podem causar poluição do ar e da água, problemas de ruído e acidentes industriais. Um dos principais objetivos deste trabalho é determinar os níveis de metais pesados (Pb, Cd, Cu, Cr, Co, Mo e Ni) nos tecidos do fígado, coxas e peito de frangos criados em torno da fábrica de cimento em uma área residencial. Determinou-se que os níveis de metais pesados no fígado de frangos criados nestes domínios são bastante elevados (P <0,01) e também, na análise de metais pesados realizada, os valores obtidos para as amostras tomadas a partir do ovo, coxa e peito das aves de capoeira são especificados como elevada (P <0,01).

Palavras-chave: ICP-AES (Espectrofotometria de emissão atômica), metal pesado, fígado, aves domésticas, sangue

Introduction

As a result of this, environmental protection consciousness has started to become evident. Besides using more productive sources, programmes regarding to environmental protection such as less consumption, reutilization of wastes are adapted within the industrialization process.

Environment is the physical, biological, social, economic and cultural media where people and other living creatures continue their relations during their lives and they stay in interaction mutually. If we cannot keep our surrounding under control, this situation will become a regretting case for us in the future. Life and environment are two important living frameworks that are bound to each other.

In this study, it is investigated whether there is any possible heavy metal pollution arising from the cement production in the chickens (thigh, chest, liver) in the eggs that are bred in the villages around the cement factory in this industrial zone or not.

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Because the civilization level varies in parallel to the energy consumed, total energy quantity consumed in the world increases and therefore the sources that are extremely required to maintain living such as air, water and soil are continuously polluted and the quantity of pollution continuously increases. This situation has reached to a threatening level for living (Akova 2008).

However in the last 30 years, discussions regarding to the noise, vibration, emissions of some gases such as sulphur dioxide, nitrogen oxides and also the possibility of the presence of heavy metals in the gases have changed the intensity and size of the problems and it is obvious that these problems are so important due in no small part (Pekin 2009).

Indexes specifying the quantities that are safe for both animals and humans are being developed. Dangerous waste units and other industrial wastes are possible one of the major pollution sources today (Henry and Miles 2001).

Because the animals are exposed to metals via food and water, it is required to know some safe value regarding to the metals in these sources. Among the metals, especially the metals such as Cu, Pb, Cd, Ni cause acute and chronic intoxication in animals. Diagnosis of chronic intoxications can be mostly possible in living animals by the analysis of urine, milk, blood, fleece, liver, kidney and carcasses (WHO 2006).

According to Bigersson et al (1988), heavy metals are classified as vital and non-vital according to their participation levels in biological processes. On the other hand, non-vital heavy metals may affect psychological structure even at very low concentrations and can cause health problems. The best example for this group is mercury bonded to sulphurous enzymes (Kahvecioğlu et al 2004a).

Heavy metals are mostly present in the soil and in the ecosystem related to water. Even if they are pretty low quantities, they can also be present in the atmosphere in the form of vapor. Metal intoxication for plants caries according to the plant type, metal type, concentration, chemical structure, soil formation and pH value and most of the metals are evaluated as essential for the growth of the plants. (Raikwar et al 2008).

It is known that heavy metal ions cause very different diseases in animals and humans, they delay the development of agricultural products and also they cause gene mutations. On the contrary of organic pollutants, heavy metal ions in the active mud in the soil, bridges and waste water purification plants cannot be eliminated by microorganisms (Shen 2003).

Air is the most important route of environmental lead (Pb) cycle. Despite this material is densely present in the air, we cannot feel it with our senses and thus it is breathed by humans and animals. Alveolar surfaces permeable for oxygen and they are risk places because they are also permeable for other contaminants (Dündar and Aslan 2005).

Although Cu is important in terms of the body functions, it is the essential component of hair, elastic sections of the skin, bone and some of the internal organs. In adults, 50-120 mg copper is present in average and it is an essential component of the reactions of amino acids, fatty acids and vitamins in metabolism under normal conditions. Copper, present in the structures of numerous enzymes and proteins, undertakes the activator duty in the functions of iron. In Cu deficiency; abnormalities, anemia, bone errors and nervous system disorders are observed in animals (Kahvecioğlu et al 2004b).

Cobalt (Co) is absolutely required as a material in the structure of especially B12 vitamin and its derivatives (Cobalamins) for animals and humans. Also it is required in the N2 binding of rhizobium (legume) and similar activities of various bacteria and spirulina. Average cobalt content of territorial crust is 18 mg/kg. Rocks consisting of high quantities of Iron (Fe) and Manganese (Mn) also include high quantities of Co. Co content in the plants is generally between 0,02 and 0.5 mg/kg and this value is higher in legume than the field plants (Gültakti 2006).

There is a close relationship between the copper (Cu) and Molybdenum (Mo) metabolism. In animal breeding, when feeding with baits consisting of low quantity of molybdenum is in question, copper accumulation in the organism accelerates. By having such metals excreted from the body very slowly, when they are continuously taken at small doses, they primarily accumulate in the liver. When its density in this organ reaches to dangerous levels, it is released into the blood circulation (Yarsan et al 1996).

Nickel (Ni) is considered to be a required trace element for some of the animals and its low concentration is accepted to have positive effects for the plants and microorganisms. However its positive effect on humans is not exactly specified yet. Daily nickel uptake per person via the foodstuff is about 0,3-0,5 mg. Ni reaches to the environment from air and then to the living creatures and the soil (Özbek 1993).

Işıklı et al (2006) have investigated the cadmium density in the soil and plant types in rural areas that are exposed to the emission of the cement factory. Despite these results show that clinical tools have no effect on the people tested except contact dermatitis, it is specified that the cement factory has increased the cadmium pollution in immediate vicinity.

In another investigation performed on the properties of the urban area soil, it is observed that urban and suburban areas are generally exposed to heavy metal and pesticide-derived pollution (Thornton 1990).

Newman (1979) has reviewed the existing information about the industry-derived air pollutants on vertebrate wild life and specified that the pollutants have caused a decrease in the bird and mammalian population in

the world. The investigator has specified the largest effects of industrial air pollution on natural life are direct death, weakness due to the industry-derived injuries and diseases, physiologic anxiety, anemia and biological accumulation and have stated that some of the air pollutants have caused changes in the distribution of some specific wild life types.

In the study of Demirbaş (1999), 11 metal (Cd, Ca, Cu, Fe, Pb, Mg, Mn, Hg, K, Na and Zn) and a non-metal (P) (heat, gaster, liver, kidneys and spleen and a little mineral material on the tissues and meats of the chickens) are specified on the chicken at different ages (4, 8 and 18 weeks) by using an atomic absorption spectrophotometer.

Paone (2008) has observed heavy metal emissions at the cement plants that are using dangerous wastes as fuel. The amendments made recently in the arrangements regarding to the emission of heavy metals, especially mercury, have caused more concern at the plants that are not using dangerous wastes.

Nicholson et al (1999) have collected and analyzed 183 bait and 85 animal manure samples from the farms in England and Galler in order to determine the heavy metal accumulations. In the analyses, Zn and Cu densities are determined as 150-2920 mg Zn/kg dry material (DM) and 18-217 mg Cu/kg DM in the pig baits depending on the ages of the animals.

In the findings obtained as a result of the investigation performed to specify that whether Pb and Cd pollution is present in the chicken meat, kidney and livers that are sold for human consumption, Pb is present in chicken meat at a ratio of <0,05 mg/kg, in liver at <0,05-0,34 mg/kg and in the kidneys at <0,05-0,2 mg/kg and Cd is present in the chicken meat at a ratio of <0,003-0,007 mg/kg, in the kidneys as <0,003-1.501mg/kg and in the liver as <0,003-1,230 mg/kg. As a result of the studies, Pb and Cd shows accumulation at a higher density in the internal organs when compared to the muscles and also it is notified that among the samples, Cd has exceeded the legal tolerance limits only in one liver and kidney samples (Sinigoj and Doganoc 2000).

Materials and Methods

This research is executed around the cement factory that shows activity at Çanakkale / TURKEY. Chickens are taken from the specified villages and quarters and they are numbered by affixing clamps. All information regarding to the study are recorded. The chickens collected are brought to the asylums in Çanakkale Onsekiz Mart University–Technological and Agricultural Research Center (ÇOMÜ-TETAM) campus. In slaughtering; thigh, chest and liver samples are placed in disposable polyethylene sampling containers and they are transferred to the laboratory with a cold chain. Thigh, chest and liver samples are stored in deep-freeze at -18^{0}C for analysis.

The samples taken in an unused plastic container are completely grinded by a plastic stirrer and a grinder and mixed homogenously. Homogenized samples (thigh, chest and liver) are weighed in glass flasks as 5 g and then 25 ml concentrated HNO_3 (nitric acid) and 10 ml H_2O_2 (hydrogen peroxide) are added and they are ignited by the method of wet decomposition in open air. The samples that are completely ignited are completed to 10 ml with purified water.

Also for control purposes, 1 control sample is prepared for every 10 samples. In the method of wet decomposition in open air, the sample temperature equipment is adjusted to 100^{0}C at first and the sample kept at this temperature for 1 hour is then waited for another 1 hour by adjusting the temperature to 130^{0}C and decomposition process has continued until the sample is exactly clarified at 150^{0}C and it becomes as 1ml. The egg samples that are completely decomposed are left for cooling and they are filtered in plastic tubes via a membrane filter and completed to 10ml with purified water and they are made ready for heavy metal analysis. Heavy metal quantities in the filtrate obtained is determined via ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometer), (Varian-Vista Model) device. (Soltanpour, P.N.; and Workman, S.M. 1981). The measurements of element concentrations are controlled by the certified values of the minerals in the reference material that is supplied from National Standards and Technology Institute (NIST, Gaithersburg, MD, USA).

Results and Discussion

The results of the Turkey multiple comparison tests performed to investigate the differentiation of the heavy metal residue levels in the chickens selected from the hencoops in the same area according to their organs and the differentiation of the heavy metal residue levels in the same organs of the chickens according to the areas are measured via the repeated measurement and observed that organ both the and the area are important for all the heavy metals (P=0,000). Thus the heavy metal quantities accumulated in the thigh, liver and chest parts of the chickens are shown in Figure 1.

When the unilateral life and consumption periods are considered, heavy metal pollutions in the products obtained from the chickens that have shorter lifetimes when compared to the other living creatures and that are used in human foods (egg, meat) are directly affected from the genotype, feeding, cultivation system and the environmental pollutions. (Holeman et al 1993; Şekeroğlu and Akmaz 2009).

The findings of this investigation has shown that the accumulation in the tissues of the heavy metals is as liver>thigh>chest like the similar investigations. This result is in parallel with the literature data and the result of the

study is supported. According to the literature data, lead taken by foodstuff is especially accumulated in the bones, blood, kidney, liver and muscle tissues and cadmium is accumulated mostly in the kidneys and then liver, blood, bone and muscles. The findings of this investigation indicate the presence of Pb and Cd in liver and muscle tissues at low levels.

Table 1 Heavy metal averages in the thigh, liver and chest tissues of the chicken (ppm).

Heavy Metals	Organ	Area 1	Area 2	Control
Pb	Thigh	0,0360 ± 0,0009 Ba	0,0266 ± 0,0004 Bb	0,0278 ± 0,0005 Bb
	Liver	0,0650 ± 0,0017 Aa	0,0374 ± 0,0005 Ac	0,0404 ± 0,0005 Ab
	Chest	0,0191 ± 0,0004 Ca	0,0165 ± 0,0002 Ca	0,0138 ± 0,0003 Ba
Cd	Thigh	0,0234 ± 0,0005 Ba	0,0238 ± 0,0003 Ba	0,0255 ± 0,0004 Aba
	Liver	0,0518 ± 0,0015 Aa	0,0338 ± 0,0003 Ab	0,0364 ± 0,0005 Ab
	Chest	0,0135 ± 0,0003 Ca	0,0150 ± 0,0002 Ca	0,0147 ± 0,0008 Ba
Cu	Thigh	0,4176 ± 0,0068 Ba	0,5243 ± 0,0071 Ba	0,5039 ± 0,0200 Ba
	Liver	1,9630 ± 0,0264 Ac	2,2121 ± 0,0487Ab	2,4896 ± 0,0827 Aa
	Chest	0,2326 ± 0,0038 Ca	0,3538 ± 0,0050 Ca	0,3353 ± 0,0158 Ba
Cr	Thigh	0,2095 ± 0,0037 Bb	0,3119 ± 0,0050 Ba	0,0622 ± 0,0066 ABc
	Liver	0,3198 ± 0,0054 Ab	0,4775 ± 0,0079 Aa	0,0983 ± 0,0118 Ac
	Chest	0,1371 ± 0,0026 Cb	0,2103 ± 0,0038 Ca	0,0371 ± 0,0041 Bc
Co	Thigh	0,0605 ± 0,0025 Bb	0,2118 ± 0,0061 Ba	0,0605 ± 0,0053 ABb
	Liver	0,0955 ± 0,0033 Ab	0,3377 ± 0,0091 Aa	0,0973 ± 0,0111 Ab
	Chest	0,0315 ± 0,0015 Cb	0,1379 ± 0,0045 Ca	0,0425 ± 0,0046 Bb
Mo	Thigh	0,0707 ± 0,0028 Bb	0,2367 ± 0,0067 Ba	0,0575 ± 0,0051 ABb
	Liver	0,1135 ± 0,0054 Ab	0,3724 ± 0,0096 Aa	0,0832 ± 0,0072 Ac
	Chest	0,0373 ± 0,0017 Cb	0,1555 ± 0,0047 Ca	0,0396 ± 0,0040 Bb
	Thigh	0,0430 ± 0,0010 Bb	0,0554 ± 0,0022 Ba	0,0276 ± 0,0008 Bc
	Liver	0,0759 ± 0,0023 Ab	0,0804 ± 0,0031 Aa	0,0429 ± 0,0012 Ac
	Chest	0,0223 ± 0,0007 Cb	0,0348 ± 0,0014 Ca	0,0157 ± 0,0006 Bc

*In the same area, differences between the organs shown with different upper cases are important (P≤0,01).
** In the same area, differences between the organs shown with different lower cases are important (P≤0,01).

Literature data notifies that the primary affected tissue by lead that is a toxic metal is bone. Thus lead mostly accumulates in the bones and 90-95% of the lead accumulated in the body is stored here (Finley 1978; Ozan 1996). Secondary affected organs by the lead are liver and kidneys. (Humphereys 1991; Roberts et al 1978). Lead that enters in the body and mixes with blood is carried by the contribution of erythrocytes and plasma albumins. Increase of the level of free or ionized lead in the blood occurs in large amount of lead uptake causing to exceed the binding capacities of erythrocyte and plasma proteins. Lead concentration in the kidneys and livers of the animals having normal lead level in the blood and no clinical intoxication symptoms may also high. Lead tends to be present for a long time in these organs (Humphereys 1991; Ozan 1996). These results are parallel to the opinions of some researchers (Bakallı 1995; Buggiani 1992; WHO 1992; Irwın 1989; Osbom 1983; Taylor and Brown 1983, Kurnaz 2008) who have findings regarding to the lead concentrations via lead uptake by foodstuff in various tissues especially in blood and bone tissues. In an experimental study of Irwin et al (1989), it is specified that lead has accumulated in bones, kidneys, livers and muscle tissues in a decreasing level in the ducks fed for 14 weeks in an artificial lake consisting of various quantities of lead particles. The study findings of Kurnaz (2008) have determined that the heavy metal accumulation level is higher in the liver tissues than the thigh tissues and they are suitable to the Turkish Food Codex and European Union regulations and these findings also support our study results.

In the measurements of Salisbury et al (1991) that are performed on the internal organs and tissues of the slaughtered chickens in Canada, it is specified that cadmium is about 0,03±0,04 µg/g (between 0,01 and 0,79µg/g) in the

livers, copper is about 3.97±0,90 µg/g (between 0,97 and 18.90 µg/g) in the livers and lead is about 0,07±0,06 µg/g (between 0,04 and 0,39 µg/g) in the livers. The findings obtained from this study also support our findings.

Conclusion

The findings of this study shows that heavy metal pollution in the samples obtained from the villages and quarters around the cement factory are under the values specified by Turkish Food Codex and European Union regulations. However rapidly constructing thermal plants and heavy industry disturb with their presence. In parallel, the companies increase their investments and growth every passing day. It will be undoubted to have the manure and chemicals used for intense agricultural reasons will have more negative effects on such negativities. While the area is being industrialized, protection and purification factors should be provided to be kept at the highest level and the observation of the changed in the environment should be continued by taking samples from the air, sea, underground and over ground water, plants, animals and humans in the region periodically.

Raising the awareness of population that is the most important condition should be provided by the families starting from the small ages and also the national education curriculum, governments, civil society organizations.

References

Akova İ (2008) Renewable Energy Sources, Nobel Publication Distribution, Ankara.

Pekin A (2009) http://www.e-kutuphane.imo.org.tr/pdf/12323.pdf.

Henry PR and Miles RD (2001) Heavy Metals – Vanadium Poultry. Ciência Animal Brasileira 2 :11-26.

WHO (2006) Protecting Groundwater for Health. Managing the Quality of Drinking-water Sources.TJ International (Ltd), Padstow, Cornwall, UK .

Bigersson B, Sterner O, Zimerson E (1988) Chemie und Gesundheit. Eine Verst 2ndliche Einführung in die Toxikologie. VCHV erlagsgeselschaft.

Kahvecioğlu Ö, Kartal G, Güven A, Timur S, (2004a) Metals Environmental Effects-I. Journal of Metallurgy 47-53.

Raikwar MK, Kumar P, Singh M, Singh A (2008) Toxic Effect of Heavy Metals in Livestock Health. Veterinary World, 1, 28-30.

Shen J (2003) "Biosorption of cupric and cadmium ions by corncob particles" (Master Thesis). Ottawa University, Canada.

Dündar Y, Aslan R (2005) The effects of heavy metals of surrounding of the life. Kocatepe Medical Journal 6:1-5.

Kahvecioğlu Ö, Kartal G, Güven A, Timur, S (2004b) Metals Environmental Effects II .Metallurgy Journal, 137:46-51.

Gültakti Y (2006) The research of heavy metal contents on some grain and legumes that grown in Van lake basin. Master Thesis. Yüzüncü Yıl University, Van.Turkey.

Yarsan E, Özdemir M, Turhan E (1996) The case of the acute poisoning with lead on cattles.

Özbek T (1993) Agrology, Cukurova University, Faculty of Agriculture. No: 458 Adana, 225.

Işıklı B, Demir TA, Akar T, Berber A, Ürer SM, Kalyoncu C, Canbek M (2006) Cadmium Exposure From The Cement Dust Emissions: A Field Study in Rural Residence. Chemosphere 63:1546-1552.

Thornton I (1990) Metal contamination of soils in urban areas. In Bullock P and Gregory PJ (ed) Soils in the urban environment. Springer-Verlag, New York.

Newman JR (1979) Effects of Industrial Air Pollution on Wildlife. Biol. Conserv 15:181-190.

Demirbaş A (1999) Proximate and Heavy Metal Composition in Chicken Meat and Tissues. Food Chemistry 67:27-31.

Paone P (2008) Heavy Metals in the Cement Industry: A Look at Volatile Cycles and Simple Mitigation Techniques. IEEE/PCA Cement Industry Technical Conference.

Nicholson FA, Chambers BJ, Williams JR, Unwin RJ (1999) Heavy Metal Contents of Livestock Feeds and Animal Manures in England and Wales. Bioresource Technology 70:23-31.

Sınıgoj-Gancnık K, Doganoc DZ (2000) Contamination of Farm Animals and Fishesfrom Slovenia With Heavy Metals and Sulfonamides Bull. Environ Contam Toxicol 64:235-241.

Holeman A, Smodis B, Anke M, Meissner D, Mills CF (1993) Heavy metal content in hens eggs. Trace elements in Man and Animals TEMA 8,Proc. 8th Int Symp. on Trace Elemants in Man and Animals, 249-250, Gersord Germany.

Şekeroğlu A and Akmaz Y (2009) The effect of the distance of the highways on the heavy metal content of the egg. Anatolia Agricultural Sciences Dergisi 24:103-107.

Fınley MT and Dıeter MP (1978) Influence of Laying on Lead Acumalation in Bone of Mallard Ducks. J Toxicology Environ Health 4:123-128.

Ozan K and Ünsal A (1996) General Toxicology. Istanbul University Veterinary Faculty Publication 48:2-70, Istanbul.

Humphereys DJ (1991) Effects of Exposure to Excessive Quantıtıes of Lead on Animals. British Veterinary Journal 147:18-30.

Roberts RD, Johnson MS, Hutton M (1978) Lead Contamination of Small Mammalsfrom Abondoned Metalliferus Mines. Environ Pollut 15: 61-69.

Bakallı RI, Pestı GM, Ragland WL, Konjufca V (1995) Dietary Copper in Excess of Nutritional Requirement Reduces Plasma and Breast Muscle Cholesterol of Baldwin DR, Marshall WJ. "Heavy Metal Poisoning and Its Laboratory Investigation" (Review Article), Annals of Clinical Biochemistry (1999); 36:267-300.

Buggıanı SS and Rındı S (1992) Lead Toxicosis and Salt Glands in Domestic Ducks. Bull. Environ. Contam. Toxicol. 24:152-155. Cadmium Environmental Aspects. Geneva.

World Health Organızation (1992) IPCS Environmental Health Criteria No: 34.

Irwin JC and Karstad LH (1989) The Toxicity for Ducks of Disintegrad Lead Shot in a Simulated-Mars Environment. Ġn: Lead –Environmental aspects. Environmental Healt Criteria 85. World Health Organization, Geneva.

Osbom D and Every WIJ, Bull KR (1983) The Toxicity of Trialkyl Lead Compounds to Birds. Environ Pollut 31:261-275.

Taylor A and Brown A (1983) Simple and Rapid Procedure fort the Determination of Lead Whole Blood by use of a Slotted Tube and Dicrete Nebulisation Flame Atomik Absorption Spectrometry. Analyst September 7:1159-1161.

Salısbury Craıg DC, Chan W, Saschenbrecker PW (1991) Multielement Schroeder H., A., 1974.The poisons around us. Toxic metals in food, air and water. Indiana University Press, Bloomington, London.

Soltanpour PN and Workman SM (1981) Use of inductively-coupled plasma spectroscopy for the simultaneous determination of macro and micronutrients in NH HCO–DPTA extracts of soils. In Barnes R.M. (ed). Developments In Atomic Plasma Analysis, USA, pp.673-680.

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