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Identification of some inorganic elements in tissues of some wild birds in Iraq

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Abstract

The object of this study was to identification of some inorganic elements in tissues of some wild birds included House sparrow, White- eared bulbul, Collared dove and Rock dove, these birds were collected from different Iraq cities during 2012. Samples of tissues included blood, liver, spleen, heart, kidney, breast and legs muscles from these birds were collected and analyzed for ash, macro-elements and micro-elements included Ca, P, Mg, Fe, K, Mn, B, and Zn. Results obtained revealed that White- eared bulbul has the high percentage of ash content in whole blood which was 4.74±0.82, also White- eared bulbul and House sparrow have the highest percentage of ash content in there kidney, breast muscle and leg muscle, whereas Collared dove and Rock dove have the lower percentage. House sparrow and White- eared Bulbul have the highest Ca percentage in whole blood and kidney which were 1023 ±85, 997 ±64 and 255 ±1.63, 257 ±1.61 respectively, House sparrow and White- eared Bulbul have the highest Fe and k percentages in whole blood and kidney compared with Collared dove and Rock dove. No significant differences were appeared in the other studied elements among studied birds and their tissues.

Key words: Inorganic elements, Tissues, Wild birds, Iraq.

Introduction

Minerals are inorganic nutrients or substances, usually required in small amounts from less than 1 to 2500 mg per day, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Although they yield no energy, they have important roles to play in many activities in the body (Malhotra, 1998; Eruvbetine, 2003).

Every form of living matter requires these inorganic elements or minerals for their normal life processes (Hays and Swenson, 1985; Ozcan, 2003). Minerals may be broadly classified as macro (major) or micro (trace) elements. The third category is the ultra trace elements. The macro-minerals include calcium, phosphorus, magnesium and sodium, while the micro-elements include iron, copper, cobalt, potassium, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulfur (Eruvbetine, 2003).

The macro-minerals are required in amounts greater than 100 mg/dl and the micro-minerals are required in amounts less than 100 mg/dl (Murray *et al.*, 2000). The ultra trace elements include boron, silicon, arsenic and nickel which have been found in animals and are believed to be essential for these animals. Evidence for requirements and essentialness of others like cadmium, lead, tin, lithium and vanadium is weak (Albion Research Notes, 1996; Soetan *et al.*, 2010).

Heavy metals occur naturally in the ecosystem with large variations in concentrations. In modern times, anthropogenic sources of heavy metals, i.e. pollutions from the activities of humans, have introduced some of these heavy metals into the ecosystem. The presence of heavy metals in the environment is of great ecological significance due

to their toxicity at certain concentrations, translocation through food chains and non biodegradability which is responsible for their accumulation in the biosphere (Abdul Jameel *et al.*, 2012). The objective of this study was to identified inorganic elements in tissues included blood, liver, spleen, heart, kidney, breast and legs muscle of some wild birds in Baghdad city as a species characteristics and pollution aspects.

Materials and Methods

Bird collection: A total of ten individuals of House sparrow (5 males and 5 females), eight of White-eared bulbul (4 males and 4 females), ten of Collared dove (5 males and 5 females) and ten of Rock dove (5 males and 5 females) were collected from different regions of Iraq during 2012.

Tissues collection: Whole blood was drawn from each bird species by bleeding method using sharply knife, released blood were collected in a 10 ml heparinized test tube. The blood samples of each bird species were pooled in one bottle and frozen until the time of analysis. Samples of liver, spleen, heart, kidney, breast and legs muscle were also collected from birds bodies using anatomical apparatus, and each organs were collected together in plastic bags and frozen until the time of analysis.

Chemical analysis: Tissues samples of all species were dried in conventional oven at 98 C for 24 hr and powdered. Ash, macro-elements: Calcium (Ca), Phosphorous (P) and Magnesium (Mg), micro-elements: Iron (Fe), Potassium (K), Boron (B), Manganese (Mn), Zinc (Zn), were determined according to AOAC (1980), all these measurements were done in triplicates. Ash determined by ashing samples using muffle furnace oven at 600 C for 6 hr. All analyzed elements were done by weighing approximately 0.5 g of sample and digested in screw-cap bottles with concentrated high purity nitric acid, bottles were heated for 6 hr and opened several times to release CO_2 buildup, digested samples were diluted to 100 ml using distilled water.

Ca and Mg determinations were done by add 10 ml of the sample solution into a 25 ml conical flask. Prepare a 0.005 mol L^{-1} EDTA solution by diluting the 0.05 mol L^{-1} EDTA solution by a factor of 1/10. Add 20mL of this diluted EDTA to the sample solution. Add 10 ml of the ammonia buffer and 1 ml of Eriochrome Black T indicator solution. Prepare a 0.0025 mol L–1 magnesium chloride solution by diluting the 0.025 mol L^{-1} magnesium chloride solution by a factor of 1/10. Titrate the sample solution with this 0.0025 mol L^{-1} magnesium chloride solution until a permanent pink color appears. Repeat the titration with further samples until concordant results (titers agreeing within 0.1 ml) are obtained. Calculate the total moles of EDTA added to the sample solution. Calculate the moles of the magnesium chloride solution used in the back titration from your concordant results. From the equation of the titration below, the moles of Mg^{2+} will be equivalent to the moles of excess EDTA. Given the ratio of Ca^{2+} and Mg^{2+} : EDTA = 1 : 1, calculation the moles of Ca2+ and Mg^{2+} that must have been complexed with EDTA by subtracting the excess EDTA from the total moles of EDTA added to the sample. This result is the moles of Ca^{2+} and Mg^{2+} in the sample solution.

K and B were determined by automatic flame photometer PGI 2000, which give the concentration in ppm. Other elements were determined by colorimetric methods using LKB Spectrophotometer.

Statistical analysis: Data were analyzed by using the General Linear Model Procedure of SAS (2001). Means were compared by the Duncan's Multiple Range test at 5% probability (Steel and Torrie, 1960).

Results and Discussion

Table (1) appeared that ash content in tissues of some wild birds were differed due to bird species, White- eared bulbul has the high percentage of ash content in whole blood which was 4.74±0.82, also White- eared bulbul and House sparrow have the highest percentage of ash content in there kidney, breast muscle and leg muscle, whereas Collared dove and Rock dove have the lower percentage.

Table (2) showed that Ca percentage in tissues was the highest percentage among the other macro-elements in all studied birds, also whole blood has the highest percentage among the other studied tissues. House sparrow and White- eared Bulbul have the highest Ca percentage in whole blood and kidney which were 1023 ±85[a], 997 ±64 and 255 ±1.63, 257 ±1.61 respectively, whereas P and Mg percentage not differ significantly among studied birds and their tissues.

Table (1): Ash content in tissues (dried) of some wild birds in Iraq (gm/100gm) ±SE.

Tissues (gm/100gm)	Wilde Birds			
	House sparrow	White- eared bulbul	Collared dove	Rock dove
Whole blood	4.56±0.85[b]	4.74±0.82[a]	4.42±0.84[b]	4.44±0.80[b]
Liver	1.45±0.38[a]	1.49±0.45[a]	1.44±0.38[b]	1.42±0.36[b]
Heart	1.21±0.43[a]	1.23±0.47[a]	1.22±0.44[a]	1.19±0.53[a]
Spleen	2.80±0.59[a]	2.83±0.65[a]	2.81±0.53[a]	2.78±0.61[a]
Kidney	3.27±0.85[ab]	3.39±0.87[a]	3.16±0.88[b]	3.18±0.88[b]
Breast muscle	1.23±0.34[a]	1.24±0.36[a]	1.19±0.33[b]	1.20±0.32[b]
Legs muscle	1.14±0.46[a]	1.16±0.45[a]	1.12±0.39[b]	1.11±0.415[b]

[a,b] different superscripts in a row differ significantly (P<0.05).

Table 2: Macro-elements content in tissues (dried) of some wild birds in Iraq (ppm) ±SE.

Tissues	Macro-elements (ppm)	House sparrow	White- eared bulbul	Collared dove	Rock dove
Whole Blood	Calcium (Ca)	1023 ±85[a]	997 ±64[ab]	978 ±80[b]	981 ±71[b]
	Phosphorous (P)	468 ±55[a]	462 ±58[a]	456 ±52[a]	453 ±56[a]
	Magnesium (Mg)	228 ±37[a]	234 ±39[a]	218 ±33[a]	219 ±38[a]
Liver	Calcium (Ca)	182 ±1.43[a]	197 ±1.47[a]	168 ±1.44[a]	175 ±1.40[a]
	Phosphorous (P)	41 ±0.62[a]	42 ±0.61[a]	41 ±0.58[a]	41 ±0.63[a]
	Magnesium (Mg)	35 ±0.35[a]	33 ±0.34[a]	32 ±0.36[a]	33 ±0.33[a]
Heart	Calcium (Ca)	227 ±0.85[a]	225 ±0.84[a]	219 ±0.86[a]	221 ±0.86[a]
	Phosphorous (P)	35 ±0.25[a]	34 ±0.22[a]	33 ±0.27[a]	34 ±0.21[a]
	Magnesium (Mg)	23 ±0.22[a]	25 ±0.23[a]	24 ±0.22[a]	23 ±0.23[a]
Spleen	Calcium (Ca)	196 ±1.58[a]	198 ±1.55[a]	194 ±1.56[a]	196 ±1.53[a]
	Phosphorous (P)	46 ±0.67[a]	46 ±0.67[a]	45 ±0.66[a]	42 ±0.69[a]
	Magnesium (Mg)	37 ±0.39[a]	36 ±0.43[a]	36 ±0.37[a]	38 ±0.41[a]
Kidney	Calcium (Ca)	255 ±1.63[a]	257 ±1.61[a]	241 ±1.64[b]	248 ±1.66[b]
	Phosphorous (P)	61 ±0.62[a]	73 ±0.61[a]	67 ±0.58[a]	65 ±0.63[a]
	Magnesium (Mg)	50 ±0.48 [a]	52 ±0.47[a]	48 ±0.47[a]	50 ±0.45[a]

[a,b] different superscripts in a row differ significantly (P<0.05).

Micro-elements percentages in tissues of studied birds were included in table (3), which appeared that House sparrow and White- eared Bulbul have the highest Fe percentage in whole blood, liver and kidney, also appeared that House sparrow and White- eared Bulbul have the highest K percentage in whole blood and kidney compared with Collared dove and Rock dove. No significant differences were appeared in the other studied elements among studied birds and their tissues.

House sparrow (*Passer domesticus*) is actually a member of the birds of Iraq (Allouse, 1962), belong to the weaver family, a large group of Old World birds. House sparrows have spread from Eurasia, and can now be found living with humankind around the globe and very common in human-made habitats (Campbell *et.al.*, 2001; BirdLife International, 2008). White-eared bulbul (*Pycnonotus leucotis*) is a member of the bulbul family. It is found in Iraq and on the Arabian peninsula nesting on the trees (BirdLife International, 2006). Collared dove (*Streptopelia decaocto*) and Rock doves (*Columba livia*) are actually members of the birds of Iraq, they have well adapted in Baghdad areas, nesting on the

Table 3: Micro-elements content in tissues of some wild birds in Iraq (ppm) ±SE.

Tissues	Micro-elements (ppm)	House sparrow	White- eared bulbul	Collared dove	Rock dove
Whole Blood	Iron (Fe)	127 ±2.77[a]	129 ±2.80[a]	117 ±2.76[b]	119 ±2.74[b]
	Potassium (K)	56 ±0.85[a]	54 ±0.85[a]	47 ±0.88[b]	45 ±0.84[b]
	Manganese (Mn)	23 ±0.30[a]	25 ±0.36[a]	24 ±0.35[a]	26 ±0.33[a]
	Boron (B)	9 ±0.12[a]	8 ±0.15[a]	7 ±0.13[a]	8 ±0.16[a]
	Zinc (Zn)	27 ±0.44[a]	23 ±0.41[a]	26 ±0.43[a]	25 ±0.45[a]
Liver	Iron (Fe)	16 ±0.60[ab]	18 ±0.62[a]	12 ±0.65[b]	13 ±0.66[b]
	Potassium (K)	10 ±0.49[a]	12 ±0.55[a]	11 ±0.52[a]	11 ±0.51[a]
	Manganese (Mn)	14 ±0.25[ab]	16 ±0.22[a]	10 ±0.26[b]	11 ±0.30[b]
	Boron (B)	4 ±0.11[a]	5 ±0.13[a]	4 ±0.13[a]	6 ±0.12[a]
	Zinc (Zn)	33 ±0.64[a]	34 ±0.65[a]	31 ±0.68[a]	31 ±0.66[a]
Heart	Iron (Fe)	13 ±0.85[a]	14 ±0.84[a]	11 ±0.86[a]	12 ±0.86[a]
	Potassium (K)	25 ±0.45[a]	28 ±0.44[a]	26 ±0.48[a]	28 ±0.42[a]
	Manganese (Mn)	9 ±0.26[a]	8 ±0.22[a]	10 ±0.25[a]	10 ±0.23[a]
	Boron (B)	5 ±0.21[a]	4 ±0.21[a]	4 ±0.21[a]	4 ±0.20[a]
	Zinc (Zn)	21 ±0.33[a]	23 ±0.30[a]	22 ±0.32[a]	21 ±0.34[a]
Spleen	Iron (Fe)	32 ±0.57[a]	34 ±0.55[a]	31 ±0.52[a]	29 ±0.56[a]
	Potassium (K)	16 ±0.43[a]	14 ±0.41[a]	16 ±0.48[a]	16 ±0.44[b]
	Manganese (Mn)	19 ±0.67[a]	18 ±0.68[a]	17 ±0.64[a]	18 ±0.67[a]
	Boron (B)	11 ±0.30[a]	13 ±0.33[a]	12 ±0.31[a]	11 ±0.33[a]
	Zinc (Zn)	44 ±0.62[a]	43 ±0.61[a]	42 ±0.64[a]	41 ±0.63[a]
Kidney	Iron (Fe)	22 ±0.35[ab]	24 ±0.34[a]	18 ±0.36[b]	19 ±0.31[b]
	Potassium (K)	37 ±0.40[a]	33 ±0.42[a]	25 ±0.42[b]	24 ±0.46[b]
	Manganese (Mn)	21 ±0.23[a]	20 ±0.20[a]	21 ±0.21[a]	22 ±0.22[a]
	Boron (B)	6 ±0.10[a]	5 ±0.10[a]	7 ±0.11[a]	8 ±0.10[a]
	Zinc (Zn)	58 ±0.38[a]	57 ±0.29[a]	62 ±0.36[a]	62 ±0.32[a]

[a,b] different superscripts in a row differ significantly (P<0.05).

top of buildings, window sills and any other place they can build a stable nest (Allouse, 1962; BirdLife International, 2006; Moudhafer *et al.*, 2006). These four species of birds are differed in there genetics, habitats and feeding, so they would have different amounts of minerals in there eggshell (Al-Obaidi *et al.*, 2012), also Miguel (2003) founded that large differences in the levels of Fe, Se, Cu, Cr, and Sr in the chicken eggshell indicated a strong influence of feed and environment.

Blood constituents can be influenced by genetic type, age, sex, infection, management, climate, and stress factors as well as by sampling protocols and methods of analysis (Bowes *et.al.*, 1989; Meluzzi *et al.*, 1992). There are limited reference biochemical values for the wild birds (Keller *et al.*, 1986; Puerta *et al.*, 1989). Whereas no significant differences in P and Mg concentrations were evidenced between males and females, sex differences for serum Ca concentrations was observed: the Ca concentrations was significantly higher in flamingos females than in males (Eren *et al.*, 2006).

Beck and Hansen (2004) reported that the onset of the sexual maturity due to increase of circulating oestrogens was correlated with changes in calcium bindings proteins, particularly in calbindin 28K, and with the calcium transport intensity by the duodenal cells. They assumed that the occupancy of the oestrogen receptors by the sexual hormones caused a rapid acceleration of the duodenal Ca assimilation. These results are in agreement with previous studies conducted in quails (Poyraz, 1988) and in mallard (Fairbrother *et al.*, 1990).

Blood calcium metabolism and the amount of ionized calcium in the plasma of birds and reptiles is ediated by parathormone (PTH), calcitonin (CT), and activated vitamin D_3 (1,25 dihydrochole-calciferol). Other hormones, such as estrogen, thyroxin, and glucagon may also influence calcium metabolism in these animals. The primary function of PTH is to maintain normal blood calcium levels by its action on bone, kidneys, and intestinal mucosa. Low blood ionized calcium stimulates the release of PTH, which results in the calcium mobilization from bone, increased calcium absorption from the intestines, and increased calcium reabsorption from the kidneys. The exact role of calcitonin in lower vertebrates is unknown, but it most likely has a physiological role opposite that of PTH. Increases in blood calcium stimulate the release of calcitonin from the ultimobranchial gland, which inhibits calcium reabsorption from bone. The active form of vitamin D_3 stimulates calcium and phosphorus absorption by the intestinal mucosa. The normal plasma concentration of calcium for most birds and reptiles ranges between 8 and 11 mg/dl. The normal plasma calcium concentration varies with species and physiologic status of the reptile and most likely other ectotherms. Herbivorous diets are often deficient in calcium and contain excessive amounts of phosphorus.

Carnivorous birds fed all meat calcium-deficient diets will also develop hypocalcemia associated with nutritional imbalances in calcium and phosphorus (Puerta *et al.*, 1989). Blood constituents can be influenced by genetic type, age, sex, infection, management, climate, and stress factors as well as by sampling protocols and methods of analysis (Bowes *et al.*, 1989; Meluzzi *et al.*, 1992).

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Heavy metals like iron, tin, copper, manganese and vanadium occur naturally in the environment and could serve as plant nutrients depending on their concentrations. Mercury, lead, cadmium, silver, chromium and many others that are indirectly distributed as a result of human activities could be very toxic even at low concentrations. These metals are non-biodegradable and can undergo global ecological circles (Ekwere and Edet, 2012).

House sparrow and White- eared Bulbul have the highest Fe percentage in whole blood, liver and kidney, this finding agreed with Blomqvist *et.al.* (1987) studied the concentrations of 10 metals (Ca, Cd, CO, Cu, Fe, Mg, Mn, Pb, V and Zn) in liver and kidney tissues of dunlin *Calidris alpha* and curlew sandpiper *Calidris ferruginea*. Significant linear correlations were found between renal and hepatic concentrations of cadmium copper, magnesium, and manganese. House sparrow and White- eared bulbul have high percentage of K in whole blood, liver and kidney because of eating high amount of fruits which are rich in K (International Food Information Council Foundation, 2011; Houston and Harper, 2008; He and macGregor, 2008).

References

Abdul Jameel, A., Sirajudeen J. and Abdul vahith R., 2012. Studies on heavy metal pollution of ground water sources between Tamilnadu and Pondicherry, India . Adv. Appl. Sci. Res., 3:424-428.

Albion Research Notes, 1996. A compilation of vital research updates on human nutrition, 5: 2, Albion Laboratories, Inc. May, (1996).

Allouse, B., 1962 . Birds of Iraq. Vol. I. (in Arabic). Al- Rabita Press, Baghdad.

Al-Obaidi, F.A., Mahdi B.I. and Al-Shadeedi S.M., 2012. Identification of inorganic elements in egg shell of some wild birds in Baghdad. Adv. Appl. Sci. Res., 3 (3):1454-1458.

AOAC, Association of Official Analytical Chemists, 1980. Official Methods of Analysis. 13th ed.,

Washington, D.C.

Beck, M.M. and Hansen K.K., 2004. Role of estrogen in avian osteoporosis. Poult. Sci., 83: 200-206.

BirdLife International, 2008. *Passer domesticus*. IUCN Red List of Threatened Species. IUCN 2008. www.iucnredlist.org

BirdLife International, *Streptopelia decaocto*. 2006. IUCN Red List of Threatened Species. IUCN 2006. www.iucnredlist.org.

BirdLife International, 2004. *Pycnonotus leucotis*. IUCN Red List of Threatened Species. IUCN2006. www.iucnredlist.org.

Blomqvist, S., Frank A. and Petersson L.R., 1987. Metals in liver and kidney tissues of autumn migrating dunlin *Calidris alpina* and curlew sandpiper *Calidris ferruginea* staging at the Baltic Sea. Ecol. Prog. Ser., 35:1-13.

Bowes, V.A., Julian R.J. and Stirtzinger T., 1989. Comparison of serum biochemical profiles of male broilers with female broilers white leghorn chickens. Can. J. Vet. Res., 53:7-11.

Campbell, R.W., N.K. Dawe, I. McTaggart-Cowan, J.M. Cooper, G.W. Kaiser, A.C. Stewart and M.C.E. McNall, 2001. The Birds of British Columbia, Volume 4: Passerines (Wood-Warblers through Old World Sparrows). UBC Press, Vancouver, BC.

Ekwere, A.S. and Edet A.,2012. Prediction of gravity anomaly from calculated densities of rocks. Adv. Appl. Sci. Res., 3(4):2059-2068.

Eren, M., Çam Y., Uyanik F. and Atalay Ö., 2006. Some blood biochemical parameters in flamingos. Revue Méd. Vét., 157(5): 277-279.

Eruvbetine, D., 2003. Canine nutrition and health. A paper presented at the seminar organized by Kensington Pharmaceuticals Nig. Ltd., Lagos on August 21, (2003).

Fairbrother, A., Craig M.A., Walker K. and O'Loughlin D., 1990. Changes in mallard *(Anasplatyrhynchos)* serum chemistry due to age, sex, and reproductive condition. J. Wild. Dis., 26: 67-77.

Hays, V.W. and Swenson M.J., 1985. Minerals and Bones. In: Dukes' Physiology of Domestic Animals, 10th ed., pp. 449-466.

He, F.J. and macGregor G.A., 2008. Beneficial effects of potassium on human health. Physiol. Plant. 133(4):725-35.

Houston, M.C. and K.J. Harper, 2008. Potassium, magnesium, and calcium: their role in both the cause and treatment of hypertension. J. Clin. Hypertens. 10 (7 suppl. 2):3-11.

International Food Information Council Foundation, 2011. Potassium and Heart Health, 1100 Connecticut avenue, NW, suite 430 Washington, DC.

Keller, P., Schulze J. and Ruedi D., 1986. Organ enzyme muster und plasma enzyme aktivitaten beim Huhn, Strauss und dem Flamingo. Schweiz. Arch. Tierheilk., 128:407-418.

Malhotra V.K., 1998. Biochemistry for Students. 10th ed., Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India.

Meluzzi, A., Primiceri G., Giordani R. and Fabris G., 1992. Determination of blood constituents reference values in broilers. Poult. Sci., 1992, 71, 337-345.

Miguel, A.M., 2003. Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. Environmental Pollution, 125:393–400.

Moudhafer, A.S., Porter R.F., Langman M., Christensen B., Schiermacker-Hansen P., Al-Jebouri S., 2006. Field Guide To The Birds of Iraq. (in Arabic). Nature of Iraq and BirdLife International Press, Baghdad.

Murray, R.K., Granner D.K., Mayes P.A. and Rodwell V.W., 2000. Harper's Biochemistry, 25th ed., McGraw-Hill, Health Profession Division, USA.

Ozcan, M., 2003. Mineral contents of some plants used as condiments in Turkey. Food Chemistry, 84: 437-440.

Poyraz, O., 1988. Tavuk, bildircin ve tavuk bildircin hibritlerine ait plazma glukoz, kolesterol ve protein düzeyleri üzerine bir arastirma. Lalahan Hay. Arast. Enst., 28: 24-41.

Puerta, M.L., Huecas V. and Garcia A.L., 1989. Hematology and blood chemistry of the Chilean flamingo. Comp. Biochem. Physiol., 94A:623-625.

SAS, 2001. SAS/STAT User's Guide for Personal Computer. Release 6.12 SAS Institute, INC., Cary, N.C., USA.

Soetan, K.O., Olaiya C.O. and Oyewole O.E., 2010. The importance of mineral elements for humans, domestic animals and plants: A review. African J. Food Sci., 4(5): 200-222.

Steel, R.G. and Torrie J.H., 1980. Principle and Procedures of Statistics. 2nd ed., McGrow-Hill Book Co., Inc, New York.