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SOME HEAVY METALS CONCENTRATION IN THE BLOOD OF HORSES, MULES AND DONKEYS AS AN INDICATOR FOR ENVIRONMENTAL POLLUTION IN ASSIUT GOVERNORATE

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ABSTRACT :

Because of the wide distribution of heavy metals throughout the earth crust, as well as the remarkable environmental pollution, it is inevitable that even small levels of these metals can be detected in all animal tissues. Some of these metals (lead, Pb and cadmium, Cd) are major contaminants and have toxic effects, whereas others such as manganese (Mn), copper (Cu), iron (Fe) and zinc (Zn) are essential for all living organisms. However, these essential metals can also be dangerous at higher concentrations. The health status of animals such as horses, mules and donkeys is directly dependent upon the chemical and biochemical nature of their feedstuffs, water and the air quality they breath. Forty- three blood samples (23 of mares, 10 of mules and 10 of she-donkeys) were collected randomly and analyzed for hematological parameters (RBCs, WBCs counts, PCV, Hb and differential leucocytic count), sodium, potassium, chloride and metals (Pb, Cd, Mn, Cu, Fe and Zn). Our results for metals concentration (mean in ppm) in blood samples revealed that their levels were: (1) in Mares, 2.028 Pb, 0.16 Cd, 2.128 Mn, 1.141 Cu, 5.17 Fe and 3.317 Zn, (2) in mules, 1.985 Pb, 0.145 Cd, 3.755 Mn, 0.785 Cu, 5.60 Fe and 4.405 Zn, and (3) in she-donkeys, 2.435 Pb, 0.525 Cd, 3.11 Mn, 1.36 Cu, 5.78 Fe and 4.76 Zn. K+ and Cl- levels showed significant increase in all investigated animals but Na+ showed significant increase only in donkeys. No significant variations of RBCs, WBCs and PCV in investigated horses and mules while donkeys showed their significant decrease. Hb content appears significantly decreased in all these investigated animals. Neutrophils and band cells showed significant decrease in donkeys and horses respectively. No significant variation in case of lymphocytes. Monocytes showed significant increase in all animals but basophil showed significant decrease only in horses and mules. Highly significant increase was observed in case of eosinophils in mules and donkeys. The correlation between our evidence of high Pb and Cd levels in different investigated animals with especial references to donkeys and that previously recorded in Feedsuffs and water at Assiut Governorate support our results and indicating environmental contamination with heavy metals especially that of major health effects like Pb and Cd.

INTRODUCTION :

The health condition of grazing animals such as horses, mules and donkeys is directly dependent upon the chemical nature of their Feedsuffs, water and the air quality they breath. In areas of more developed industrial activities and due to civilization, these animals may be exposed to pollutants and thereby provide an additional source of chemical pollutants which directly affect the physiological processes of the affected animals. Upper Egypt nowadays shows numerous and growing industrialization which did not exit before 40 years ago. Moreover, in Assiut Governorate the motor vehicles increased 800 % in the same time the roads still remained narrow consequently pollution came to all areas especially that found around the main roads of this Governorate.

Heavy metals are invaluable and unavoidable components of our environment. The amount of different heavy metals in ambient atmosphere have been increasing with the development of human activities and are likely to increase further with increasing exploitation of geological resources, such as mining and fossil fuel development. An additional concern about metals in their presence and concentrations in domestic and industrial waste products, as the individual elements are indestructible [1]. Through the years considerable amounts of Pb have been mobilized into the environ-ment. Industrial smelters, discarded batteries, burning of garbage and old paint wood are the main sources of environmental Pb. Burning of coal and the foil oil constitute a source of Pb that calls for particular consideration is Pb tetra alkyl used as petrol (gasoline) additive. The Pb derived from petrol additives contributes not only to the intake through inhalation but also to the intake through ingestion as a result of fallout from vehicle exhaust on nearby food crops [2].

Heavy metals contamination of agricultural lands adjacent to highways has long been recognized especially for Pb as a result of the dispersion of motor vehicles exhaust emissions [3&4]. Pastures contaminated by smelters are recorded as carrying 325 ppm Pb in dry matter [5], while Sharkawy [3] recorded that Pb levels in Feedsuffs were 105, 135, 14.4, 84.4, 55.4 and 99.5 ppm in unwashed samples of 70 days growing beans, 110 days growing bean,, grass, barseem, wheat straw and tibn respectively. In high contaminated areas, grasses may contain 20 - 60 ppm Pb [2]. Many toxic substances, such as Pb and Cd, have always occurred in the biosphere, and a certain level of exposure must be considered inevitable as part of natural conditions for life on this planet. The anthropogenic release of Cd to the atmosphere is about 20 fold above estimated emissions from natural sources and Pb pollution is much more extensive [6]. Also, increasing environmental deposition of lead has been implicated by analysis of environmental samples that reflect both past and present conditions [7].

Manganese is considered to be an essential element for all living animals. Dietary Mn deficiency can result in a wide variety of structural and functional defects. A relationship between Mn and carbohydrate metabolism is now well recognized, while Mn is also a cofactor for a number of enzymatic reactions, particularly those involved in phosphorylation, and fatty acid synthesis [8,9&10]. On the other hand, Mn toxicity represents a serious health hazard in humans. Toxic intake of Mn (either through the air or diet) may result in severe pathological changes particularly in the CNS, neural damage, reproductive and immune system dysfunction, nephritis, testicular damage, pancreatitis and hepatic damage [11,12&13]. Copper is an essential trace element, a normal constituent of animal tissues and fluids, crucial in hemoglobin synthesis and other enzymes functions. Both deficiency and excess of copper in the mammalian system result in untoward effects [14].

Therefore, the early discovering of metallic poisoning and subclinical cases is more important, the aim of this study is to estimate lead, cadmium and manganese levels and their relative elements (copper, iron, zinc, sodium, and potassium) and chloride ions as well as haematological parameters in horses, mules and donkeys as the first step for evaluation of pollution problem at Assiut Governorate.

MATERIALS AND METHODS :

Materials:

A total number of 43 blood samples (23 from mares, 10 from mules and 10 from she-donkeys) were collected randomly from Assiut Governorate. Each blood sample was divided into two portions, one with anticoagulant for haemogram and metal estimation, and the other portion for serum.

A-Whole blood samples with anticoagulant :

Two ml of blood were collected by means of vein puncture into a clean dry sterile vials containing EDTA (Ethylene diamine tetra acetic acid) in a concentration of 1 mg for each ml of blood. The samples were mixed with anticoagulant by gentile inversion of the vials for several time [15]. These samples were used for haematology that included total erythrocytic counts (RBCs) packed cell volume (PCV), haemoglobin concentration (Hb), total leucocytic counts (WBCs), and metal estimation (lead, Pb; cadmium, Cd; manganese, Mn; copper, Cu and zinc; Zn).

B- Blood serum samples :

Ten ml whole blood samples were collected in a clean dry sterile centrifuge tube of 15 ml capacity. The blood was allowed flow freely and gently from the jugular vein over the inner surface of the tube. The samples were left for 30 minutes at room temperature to form clot then cooled in the refrigerator and centrifuged at 3000 rpm for 30 minutes [15]. The obtained blood sera were clear and free from haemolysis and kept into a clean dry sterile glass vials. These serum samples were used for determination of iron, sodium, potassium and chloride levels.

Methods:

1- Haematological picture:

Total erythrocytic count (T/L, Tera/liter $=10^{12}$/L.), total leucocytic count (G/L, Giga/liter$=10^{9}$/L.) and haemoglobin concentration (g/dl) were determined by using electronic blood cell counter (Cell Dyne, 300 sequoiturner). Packed cell volume was determined by mean of microhaematocrite method (using microhaematocrite centrifuge type 346) according to Schalm [16]. Stained blood smears (Giemsa stain) were examined for differential leucocytic counts as described by John [17].

2-Estimation of sodium, potassium and chloride:

Serum sodium and potassium were determined by flame photometer (Corning 400) while chloride was determined by using chloride analyzer (Model 925 Corning) according to Schmidt [18]

3. Estimation of metals :

1 ml of blood was digested by using a mixture of $HClO_4$-HNO_3. Lead, cadmium, manganese, copper and zinc were determined in blood while iron was measured in serum by using atomic absorption spectrophotometer (GBC 906 AA).

4- Statistical analysis :

Student's "t" test was used to calculate the significance between normal control animals (background control) and investigated animals. The haematological and blood electrolytes control values were according to Rateb [19], while control values of metals were according to Underwood [20], Ostrowski [21] and Ward and Savage [22]. Probability values 0.05 and 0.001 were considered statistically significant and this according to Kalton [23].

RESULTS :

In table 1, Cd, Mn, and Fe showed highly significant increase in all investigated animals, but Pb showed significant increase in donkeys and highly significant increase in horses and mules. In case of Cu, no significant variations in mules but there is significant increase in donkeys and highly significant increase in horses. Zn levels showed significant decrease in mules and highly significant decrease in horses and donkeys. In table 2, K+ levels showed significant increase while Cl- showed highly significant increase in all investigated animals but Na+ showed significant increase only in donkeys. In table 3, no significant variations of RBCs, WBCs and PCV in investigated horses and mules while donkeys showed significant decrease in WBCs and PCV and highly significant decrease in RBCs count. Hb content appears significantly decreased in horses and mules and highly significantly decreased in donkeys. In table 4, Neutrophil and band cells showed significant decrease in donkeys and horses respectively. No significant variation in case of lymphocytes. Monocytes showed significant increase in horses and mules but highly significantly increased in donkeys. Highly significant increase was observed in case of eosinophils in mules and donkeys. Basophil showed significant decrease in mules and highly significant decrease in horses but no significant variations in donkeys.

Table (1) : Heavy metals concentrations (ppm) in the blood of investigated horses, mules and donkeys at Assiut Governorate.

Animal	Pb	Cd	Mn	Cu	Fe	Zn
Control n=8	0.050 ± 0.001	0.001± .0001	0.020 ± 0.001	0.800±0.009	1.49 ± 0.44	7.210 ± 0.503
Horses n=23	2.028±0.169**	0.160 ±0.013**	2.128±0.065**	1.141±0.029**	5.17±0.30**	3.317± 0.046**
Mules n=10	1.985±0.320**	0.145±0.025**	3.755±0.192**	0.785± 0.034	5.60±0.37**	4.405 ± 0.801*
Donkeys n=10	2.435±0.642*	0.525±0.051**	3.110±0.134**	1.360± 0.263*	5.78±0.67**	4.760 ±0.292**

Table (2): Na+, K+ and Cl- levels (mmol/L) in the blood of investigated horses, mules and donkeys at Assiut Governorate.

Animal	Na+	K+	Cl-
Control (n = 8)	139.0 ± 3.50	3.510 ± 0.570	104 ± 2.60
Horses (n = 23)	139.5 ± 2.92	4.695 ± 0.093*	133 ± 3.22**
Mules (n = 10)	143.3 ± 3.16	5.110 ± 0.175*	137 ± 6.01**
Donkeys (n = 10)	149.8 ± 1.38*	4.930 ± 0.117*	122 ± 3.55**

Table (3): RBCs, WBCs counts, PCV and Hb levels of investigated horses, mules and donkeys at Assiut Governorate.

Animal	RBCs (T/L)	WBCs (G/L)	PCV (%)	Hb (g/dl)
Control n=8	11.580 ± 0.632	16.200 ± 1.347	43.410 ± 2.507	16.71 ± 0.607
Horses n=23	11.452 ± 0.406	15.313 ± 0.637	45.173 ± 1.095	14.66 ± 0.361*
Mules n=10	9.990 ± 0.493	15.330 ± 1.043	47.930 ± 1.516	14.87 ± 0.629*
Donkeys n=10	7.470 ± 0.299**	12.950 ± 0.266*	33.000 ± 0.969*	11.14 ± 0.266**

Table (4): Differential leucocytic counts of investigated horses, mules and donkeys at Assiut Governorate.

Animal	Neutrophils	Band cells	Lymphocytes	Monocytes	Eosinophils	Basophils
Control n=8	54.000±0.999	5.000±0.256	35.800±1.710	2.500±0.164	2.700± 0.297	0.800±0.132
Horses n=23	55.521±0.644	3.608±0.293*	33.434±0.632	3.565±0.273*	4.086±0.245	0.217±0.086**
Mules n=10	53.700±1.200	4.200±0.394	33.100±0.740	3.600±0.379*	5.200±0.368**	0.200±0.126*
Donkeys n=10	48.700±0.949*	4.400±0.322	35.500±0.724	4.900±0.330**	6.000±0.447**	0.500±0.158

- The obtained results were mean ± SE

***: Significantly different from control at $p < 0.05$.**

****: Significantly different from control at $p < 0.001$.**

DISCUSSION :

Equines (horses, mules and donkeys) are among the most sensitive of the domestic species to increased body burdens of Pb. Clinical signs of Pb intoxication in horses include laryngeal paralysis with dyspnea (roaring) and subsequent aspiration pneumonia as well as enlarged joints, stiffness, clumsiness, peripheral neuropathies, muscular weakness and anorexia [24,25&26]. In a 1971 study of 193 blood samples from normal young and adult horses in North America revealed that lead blood level was approximately 0.11 ppm, however, few studies performed after gasoline tetraethyl Pb additives were reduced to a value near 0.05 ppm which may be appropriate [26&27]. Levels greater than 0.3 ppm are usually considered indicative of toxicity, and levels of 0.5 to 0.7 ppm have been associated with severe clinical signs, often leading to euthonasia of the affected horses, although occasional horses have been reported as asymptomatic with 0.7 to 0.9 ppm [27,28,29&30]. Ostrowski [21] found lead blood levels of 0.17 in examined donkeys and mules near a secondary Pb smelter.

An excess of a given metal through dietary, occupational, or environmental exposure may lead to depletion or repletion of an essential metal at numerous biological levels: at molecular, cellular, tissue or organ, and systemic levels of organization. Cd intoxication produces necrosis in the intestine, which is prevented by sufficient Zn. Our obtained results (decrease in Zn and increase in Fe) are in accordance with Pounds [31] who stated that Pb intoxication alters tissue levels of many essential elements including Fe, Zn, Cu and Ca.

In case of Zn, our results which showed significant decrease are in accordance with Niklowitz and Yeager [32] who reported that Pb displace Zn or prevent its uptake by the brain. In their study, they showed that rabbits exposed to toxic levels of tetraethyl Pb lost 0.5 molecule Zn from the brain for each molecule of Pb they retained, and also with that obtained by Cerklewski and Forbes [33]. They found that increased dietary Zn impairs intestinal absorption of Pb in rats and thus protected from dietary Pb. Aminolevulinic acid dehydratase enzyme included in the heme synthesis is a Zn-dependent enzyme. The inhibition of the enzyme by Pb is apparently alleviated by Zn [34].

Zinc has also been reported to provide some protection to horses grazed on pasture contaminated with Pb and Zn from refinery effluent. Although their tissue content of Pb was nearly doubled, they showed fewer signs of intoxication Willioughby [29] than animals exposed to Pb alone.

The increase of Cu concentrations in the blood of investigated animals especially horses and donkeys illustrates the interference of elements during intestinal absorption. Zn hinders Pb absorption as demonstrated in the horse [29]; Zn and Pb may hinder Cu absorption [35&36]. The antagonistic effects of Zn against Cu and Fe can result in suppression of haematopioesis [37] and this explain the decrease in Hb content in all our investigated animals and the significant decrease in RBCs count in donkeys.

The obtained results in this study revealed a positive correlation between Pb and Fe in all investigated blood samples in horses, mules and donkeys. These results

are in agreement with that obtained in cattle by Sharkawy [3]. This fact of Fe increase was attributed to the inhibition of delta aminolevulinic acid dehydratase and ferrochelatase enzymes of the heme synthesis pathway which are a Zn-dependent enzymes, so that inhibition of these enzymes by Pb, result in accumulation of Fe in blood and other tissues [34]. Pb can displace Fe from tissues, rabbits given tetraethyl Pb have been observed to lose 2 molecule Fe from the brain for each molecule of Pb they retained [32].

An adverse effect of Pb on Cu metabolism is implied by the decrease in serum ceruloplasmin that occurred in rats exposed to Pb and fed adequate levels of Cu. Cu deficiency accentuated the toxic effects of Pb and Pb accumulated in the liver and kidneys. Anemia was also present and growth was retarded [38]. Our results are in agreement with the previous conclusion which appear clearly by decrease in Hb level in all investigated animals with high significant decrease in donkeys. This finding could be explained by the poor Feedstuffs admitted to donkeys that may contain high levels of Pb.

In rural areas, levels of Pb in air of 0.1 $\mu g/m^3$ or less are found. However, depending upon the degree of pollution due to urbanization, the amount of lead in city air range from 1-3 $\mu g/m^3$ and will occasionally be much higher under peak traffic conditions. On the basis of the information available and depending upon the degree of urbanization of the area concerned, its topographical situation, weather conditions and habitats, it may be assumed that the intake of lead by inhalation in cities could on occasion be 100 μg/day [2].

Zinc and Cd are known antagonists to each other [20&39]. Cd toxicity, which has similarities with Zn deficiency, can be diminished with the addition of Zn [40]. Tissue Zn may be elevated with Cd toxicity [40] and this is differ from our obtained results while Roberts [41] support our results in their study when calves fed 350 ppm Cd resulted in decrease Zn absorption.

Lead interferes with sodium reabsorption by the kidneys [42], and impair the energy dependent mechanism of RBCs for controlling sodium and potassium exchange [43]. The results in this study are different from that recorded by Sharkawy [3], who carried out his study on cattle blood.

CONCLUSION :

According to eating habits of equines (horses, mules and donkey) as they eat barseem, grasses, hay, tibn (wheat straw and bean straw), cereals and grains, and to the results obtained by Sharkawy [3] in Assiut Governorate, the elevation of Pb in investigated blood samples may return to either (1) Pb comes from Feedstuffs, or to (2) Pb comes from drinking water, or to (3) Pb content in air they breath, or from all (1, 2 and 3).

Also, high Cd levels may be due to high Cd content in Feedstuffs, cereals and grains, water admitted to these animals and the air they breath. Mumma [44] reported that cereals and grains contain from 0.01 to 0.6 ppm Cd and Mehennaoui [45] stated that non polluted and polluted hay contain < 0.05 and 4.2 ppm Cd, and this may the original source of Cd to these animals.

Although both Pb and Cd are considered prior pollutants, Pb would

appear to be a more widespread hazard with current exposure, being 10 - 100 times above the permissible limits. On the assumption that the animals bodies have limited tolerance toward increments in exposure to these toxic metals, a goal for preventive efforts would be that anthropogenic release should not add additional significant amount to the original background levels. Although variations seem to occur from place to place and from locality to locality and with dietary habits, natural exposure levels may provide a useful guide and good reference, in particular with regard to lead.

The correlation between our evidence of high Pb and Cd levels in different investigated animals with especial references to donkeys and that previously recorded in Feedstuffs and water at Assiut Governorate support our results and indicating environmental contamination with heavy metals especially that of major health effects like Pb and Cd.

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تركيز المعادن الثقيلة فى دم الخيول والبغال والحمير كمؤشر للتلوث البيئى فى محافظة أسيوط

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بالرغم من الانتشار الواسع للمعادن الثقيلة فى القشرة الارضية والتلوث البيئى الملحوظ فإنه من المُشَـاهَد أنه يمكن الكشف عن كميات صغيرة من المعادن الثقيلة فى جميع الأنسجة الحيوانية. ويُعد الرصاص والكادميوم من الملوثات الكبرى ، والتى لها تأثيرات سامة بينما يُعتبر المنجنيز والنحاس والحديد والزنك مـــن العناصر اللازمة لكل الكائنات الحية ، ولكن هذه العناصر الهامة يمكن أن تكون سامةً إذا وُجِدت بكميات كبيرة فى أنسجة الحيوانات . إن الحالة الصحية لحيوانات النقل ممثلةً فى الخيول والبغال والحمير تعتمد اعتماداً كلياً على الناحية الكيميائية لموادها الغذائية والماء وكذلك الهواء الذى تتنفسهُ ، جُمعت ٤٣ عينة دم (٢٣ أفراس ، ١٠ بغـال ، ١٠ إناث الحمير) بصورةٍ عشوائيةٍ ، وحُللت لبيان المتغيرات الدموية (عدد كرات الدم الحمراء والبيضاء ونسبة الهيموجلوبين ، وكذلك العد النوعى لكرات الدم البيضـاء) والصوديـوم والبوتاسـيوم والكلوريـد والمعـادن (الرصاص والكادميوم والمنجنيز والنحاس والحديد والزنك).

وقد أظهرت نتائج تحليل عينات الدم بالنسبة لمستويات العناصر (الرصاص والكادميوم والمنجنيز والنحـاس والحديد والزنك) مقاسة بوحدة جزء فى المليون أنه:-

١- **فى *الافراس***: مستوى كل من الرصاص ٢,٠٢٨، والكادميوم ٠,١٦ ، والمنجنيز ٢,١٢٨، والنحاس ١,١٤١ ، والحديد ٥,١٧ ، والزنك ٣,٣١٧ .

٢- **فى *البغال***: مستوى كل من الرصاص ١,٩٨٥، والكادميوم ٠,١٤٥، والمنجنيز ٣,٧٥٥، والنحـاس ٠,٧٨٥ ، والحديد ٥,٦٠، والزنك ٤,٤٠٥.

٣- **فى *إناث الحمير***: مستوى كل من الرصاص ٢,٤٣٥، والكادميوم ٠,٥٢٥، والمنجنيز ٣,١١، والنحاس ١,٣٦، والحديد ٥,٧٨ ، والزنك ٤,٧٦.

مما سبق وبعد مناقشة النتائج تبين أن هناك زيادة كبيرة فى عنصرى الرصاص والكادميوم فى جميع عينات الدم التى تم فحصها مع وجود زيادة فى عنصر المنجنيز فى عينات دم البغال . وبمقارنة النتـائج بمـا سـبق الحصول عليه فى نتائج سابقة من حيث احتواء المواد الغذائية والحبوب على عناصر ثقيلـة مثـل الرصـاص والكادميوم تبين وجود علاقة طردية بين مستويات هذه العناصر فى الأعلاف والمواد الغذائيـة المقدمـة لـهذه الحيوانات وبين مستوياتها فى دم هذه الحيوانات ، وبالتالى فى أنسجتها مما يؤثر على صحتها .

يتضح من البحث زيادة مستوى المعادن الثقيلة فى دم الحيوانات التى تم فحصها. ينصح الباحث بالعمل على اتباع كافة الوسائل الممكنة للحفاظ على البيئة فى محافظة أسيوط.