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MONITORING OF HEAVY METALS AND HEMATOBIOCHEMICAL CHANGE IN MILK AND BLOOD OF BUFFALOES FED ON SEWAGE IRRIGATED GREEN DARAMA

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ABSTRACT

The water pollution considered as a very critical environmental problem concern all over the world. The present study was planned to investigate the levels of five different heavy metals (Al, Pb, Cd, Cu and Zn) and their hemato-biochemical effects in blood of dairy buffaloes fed on sewage irrigated plant (Darawa) as well as its residues in milk. Blood and milk were sampled from two groups of dairy buffaloes (n=10 each) the first fed on Darawa where irrigation was carried out by the River Nile water (control) and the second fed on sewage irrigated Darawa in Tal-Mohamed village in Sharkia Province (exposed). Darawa allowed for these animals was also sampled. The first blood samples for hematological analysis revealed significant reduction of total erythrocytic count, hemoglobin and packed cell volume, while total leucocytic count showed insignificant increase. Serum biochemical analysis (the second blood samples) revealed significant increase of serum AST, ALT, AP, BUN, Al, Pb and Cd. On the other hand, glucose, total protein, albumin, Cu, and Zn showed significant reduction. Levels of estimated heavy metals in milk samples collected from exposed buffaloes showed significant increase when compared with those from control ones.Furthermore,the results showed that polluted plant(Darawa 2) contained higher concentrations of heavy metals than the normal plant (Darawa 1). It was concluded that, animals fed on sewage polluted plants accumulate higher metals in their blood and milk than those fed on non polluted plants and that may pose potential environmental and health risks in the long-term.

INTRODUCTION

Environmental pollution is one of the most deleterious agents to the biological life, and water pollution considered a very critical environmental problem facing the public health officials ***(Ali, 2004)***. The used water of a community is called wastewater or sewage. If it is not treated before being discharged into water ways, serious pollution is the result.

Wastewater is any water that has been adversely affected in quality by anthropogenic influence.It comprises liquid waste discharged by domestic residences, commercial properties, industry, and/or agriculture and can encompass a wide range of potential contaminants and its concentrations.

Heavy metals are natural components of the Earth's crust, but in recent years industrial, agricultural and zootechnic development have been responsible for the diffusion of these substances in the environment causing pollution of water, soil and atmosphere ***(Miranda et al., 2000).*** In general, heavy metals are not biodegradable, have long biological half-lives and have the potential for accumulation in the different body organs leading to unwanted side effects ***(Jarup, 2003; Sathawara et al., 2004 and Eslami, et al., 2007).***

Although the concentration of heavy metals in sewage effluents are low,long term use of these wastewaters on agricultural lands often results in the build-up of the elevated levels of these metals in soils ***(Rattan et al., 2001).*** Crops raised on the metal contaminated soils accumulate metals in quantities excessive enough to cause clinical problems both to animals and human beings consuming these metal rich plants ***(Tiller, 1986 and Azita & Seid, 2008).***

Milk and milk products represent and important part of the human food and so, the contamination of milk by heavy metals is one of the major threats confronting the public health ***(Jensen, 1995)***. Therefore, the animals receive the heavy metals through the ingestion of contaminated feed stuffs are considered as the main source of metal residues in the secreted milk ***(Vidovic et al., 2005).***

The object of this study is to determine heavy metals, lead, cadmium, aluminium, copper and zinc in selected green vegetable (Darawa), blood and milk of buffaloes fed on sewage irrigated plant.

MATERIALS AND METHODS

1- Animals:

Two groups of lactating dairy buffaloes (10 each), aged 4-6 years from Tal–Mohamed village in Sharkia Province were used in this study.

The first group (clinically healthy control) was selected from animals fed on Darawa irrigated with Nile River water; while, the second group (exposed) was selected from animals fed on darawa irrigated with sewage polluted water.

2- Sampling:

A- Blood samples:

Whole blood with and without anticoagulant were collected from jugular vein of the healthy and exposed buffaloes. Samples were used for hematological examination and biochemical assay.

Hematological analysis:

RBCs,Hb,PCV and WBCs were determined according to ***Jain (2000).***

Biochemical serum analysis:

Each clear serum sample was divided into 2 parts, the first part was used for determination of AST and ALT ***(Reitman and Frankel, 1957),*** ALP ***(Bowers and McComb, 1975)***, glucose ***(Lott, 1975)***, total protein ***(Doumas et al., 1981)***, albumin ***(Bauer, 1982)***, Urea ***(Patton and Crouch, 1977)*** and creatinine ***(Thomas, 1992 a).*** The second part was prepared to determine the concentrations of lead, cadmium, aluminium, copper and zinc using ***AOAC (2000).*** Atomic Absorption Spectrophotometer in Central Veterinary Laboratory, Zagazig Universty.

B- Milk Samples:

Milk samples (20 ml, each) were collected from 10 buffaloes fed on Darawa irrigated with Nile River water (control buffalues) and 10 buffaloes fed on Darawa irrigated with sewage polluted water (exposed buffaloes). Milk sample from each animal was collected in a sterile Macarteny bottle for chemical examination. Each sample was labeled to identify the source, site and date of sampling and stored in deep freeze till analysis.

Chemical analysis of raw milk:

Raw milk samples were digested according to method described by ***Tsoumbaris and Papadopoulou (1994)*** where 10 ml solution of concentrated nitric acid and perchloric acid (1:1) were added to 10 ml of thoroughly homogenized milk sample. The samples were cold digested over night followed by mild increase in temperature till heating at 100 ^{o}C in water bath for 3-4 h. Four to five drops of H_2O_2 (30%) were added and heating continue till the brown nitrous gasses were expelled and the specimens became clear. After cooling, each digested sample was diluted to 25 ml with deionized water and filtered through Whatman filter paper No. 42. The clear filtrate of each sample was kept in refrigerator to avaid evapor-ation. The filtrated samples were analyzed for trace elements under the present investigation, using Perkia-Elmer Atomic Absorption Spectrophotometer.

C- Plant samples:

Random samples of Darawa were collected from two areas in Sharkia Governorate (according to the purity of irrigated water). Plant samples were dried and Ashed ***(AOAC, 2000)*** and stored in deep freeze until biochemical analysis. Metals in plant ash were determined using Atomic Absorption by Spectrophotometer.

D- Statistical Analysis:

Data obtained were statistically analysed according to ***Snedecor and Cochran (1982)*** using student's "t" test.

RESULTS

All obtained results were recorded in Tables 1, 2, 3 and 4.

Table (1): Hematological values in healthy and exposed buffaloes.

Parameters	Healthy buffaloes	Exposed buffaloes
RBCs (x10^6/mm^3)	7.02 ± 0.19	6.28 ± 0.25*
Hb (gm/dl)	13.32 ± 0.39	11.4 ± 0.53*
BCV (%)	32.22 ± 0.87	29.96 ± 0.42*
WBCs (x 10^3/mm3)	6.68 ± 0.41	7.42 ± 0.27

*Significant at ($P<0.05$)

Table (2): Serum biochemical values in healthy and exposed buffaloes.

Parameters	Healthy buffaloes	Exposed buffaloes
AST (IU/L	59.5 ± 1.23	69.22 ± 2.2**
ALT (IU/L)	37.58 ± 1.3	49.34 ± 3.72*
AP (IU/L)	167.56 ± 1.63	181.94 ± 5.13*
Urea (mg %)	15.23 ± 0.55	23.98 ± 1.67**
Creatinine (mg %)	1.44 ± 0.13	1.6 ± 0.11
Glucose (mg %)	52.23 ± 1.24	43.96 ± 3.73*
Total protein (gm %)	7.16 ± 0.19	6.24 ± 0.23*
Albumin (gm %)	3.13 ± 0.14	2.7 ± 0.12*
Al (ppm)	0.082 ± 0.007	0.128 ± 0.011**
Pb (ppm)	0.12 ± 0.01	0.17 ± 0.009**
Cd (ppm)	0.298 ± 0.02	0.42 ± 0.03**
Cu (ppm)	1.29 ± 0.11	0.97 ± 0.03*
Zn (ppm)	1.16 ± 0.09	0.85 ± 0.07*

*Significant at ($P<0.05$) **Highly significant at ($P<0.01$)

Table (3): Heavy metals residue in milk of healthy and exposed buffaloes.

Parameters	Healthy buffaloes	Exposed buffaloes
Al (ppm)	0.052 ± 0.004	0.078 ± 0.01*
Pb (ppm)	0.2 ± 0.01	0.27 ± 0.02*
Cd (ppm)	0.005 ± 0.0004	0.007 ± 0.0004*
Cu (ppm)	0.07 ± 0.006	0.09 ± 0.004*
Zn (ppm)	0.94 ± 0.07	1.2 ± 0.04*

*Significant at ($P<0.05$)

Table (4): Concentration of heavy metals (ppm) in Darawa from normal (Darawa) and exposed (Darawa 2) areas.

Plant	AL	Pb	Cd	Cu	Zn
Darawa 1	0.7	1.3	0.19	11.3	36.5
Darawa 2	3.6	17.4	2.03	22.25	45.7

DISCUSSION

The use of sewage sludge or waste water for irrigation of agricultural lands is on the rise particularly in developing countries. Heavy metals present in sewage pose the risk of human or phytotoxicity from land application long-term use of sewage, heavy metals can accumulate to phytotoxic levels and results in reduced plants growth and/or enhanced metal concentrations in plants, which consumed by animals then enter the food chain. The effects of heavy metals can ranged from subtle symptoms to serious diseases. The worst part about heavy metals is that once build up in human body and can cause irreversible damage.

The hematological observation in Table (1) revealed significant decrease in values of RBCs, Hb and PCV while, WBCs showed insignificant increase, our results nearly coincide with those reported by ***Naser et al. (1996); Thomas (2002 b); Anwaar and Hafez (2006) and Emam & El. Nabrawy (2008)***. The observed anemic condition could be attributed to the failure of bone marrow to produce enough erythrocytes in response to the toxic effect of both lead and cadmium or due to the direct hemolytic effect on mature red blood cells ***(Moore et al., 1980).*** Also, this probably due to impaired intestinal absorption of iron due to competition of cadmium with iron transfer system from the intestinal mucosa leading to iron deficiency ***(Fox et al., 1971).*** Other explanation was offered by ***Kundomal et al. (1986),*** that the decrease in circulating erythrocytes was attributed to the indirect inhibitory effects of lead and cadmium to the heme-biosynthesis by inhibition of delta amino-levulinic acid dehydrase enzyme activity.

A signifiant increase was observed in the activity of the serum AST, ALT and AP. These results are supported by those obtained previously by; ***Omaran (1997)*** and ***Osuna et al. (2005).*** Such elevation may be referred to the destructive and toxic effects of heavy metals on liver and kidney tissues and skeletal muscles **(Oser, 1979).**

The obtained results for blood urea nitrogen and creatinine revealed significant increase of urea nitrogen while creatinine showed insignificant increase**. *George & Duncan (2005) Anwaar & Hafez (2006)*** and ***Emam & El Nabrawy (2008)*** recorded the same results for urea nitrogen. Such elevation may be due to toxic effect of lead and cadmium on kindney causing renal dysfunction and decreasing renal clearance of blood from urea ***(Sakurai et al. 1980). Hilhaud et al., (1979)*** mentioned that blood urea and creatinine were within the normal range in the early stages of cadmium exposure and gradually increased by time.

Regarding serum glucose, the results reveated significant reduction, the obtained results seemed to be in full agreement with those recorded by ***Naser et al.(1996);Anwaar&Hafez(2006); Kamel (2007) and Emam & El Nabrawy (2008)***. This reduction may be attributed to a state of inappetence and decreased food intake. ***Doxey (1971)*** reported that normal ruminants have low blood glucose levels and any slight decrease is quit sufficient to push them into a hypoglycemic state.

The present study revealed a significant decrease of serum total protein and albumin. Our results agreed with those reported by ***Harper (2002); Radostits et al. (2002)*** and ***Emam & El Nabrawy (2008)*** they mentioned that hypoproteinemia and hypoalbuminemia may be due to the toxic effect of heavy metals on the liver cells leading to impaired synthesis of protein and albumin. ***Coles (1986)*** attributed the reduction to the male-absorption of dietary content from intestinal tract.

The results of present study showed that the levels of Al., Pb and Cd were higher in blood of buffabes fed on plant irrigated with sewage wastewater than those fed on non polluted plant. Blood levels of heavy metals are broadly reflecting amounts of these elements in the food ***(Thornton, 2002 and Linzon, 2005).*** The authors also found that the ruminants especially cattle and buffaloes were commonly affected with chronic lead and cadmium poisoning due to their ability to settle-down the heavy metals containing particles in the reticulum and be converted to soluble compounds by the action of the acid medium of the forestomach and lastly enter to blood streem.

In the current work, the mean values of Cu and Zn were significantly decrease in exposed buffaloes when compared with those of control ones. The obtained results agreed with those recorded by ***Eman et al. (2008)*** which found that plasma Cu was negatively correlated with blood Cd. The explanation is that the two metals compete for binding sites on metallothionein and Cu displaces Cd from the binding sites because its higher affinity for the protein ***(Funk et al., 1987). Endo et al. (1997)*** reported that there was negative correlation between Cd and Zn due to their competition on the same binding sites.

Statistical analysis of the obtained date in (Table 3) revealed that the concentration of heavy metals (Al, Pb, Cd, Cu and Zn) in milk samples obtained from buffaloes fed on Darawa irrigated with Nile River water were 0.052, 0.2, 0.005 , 0.07 and 0.94 ppm, respectively. While, the concentration of the same heavy metals in milk samples obtained from buffaloes fed on Darawa irrigated with sewage polluted water were 0.078, 0.27, 0.007, 0.09 and 1.2 ppm, respectively. The higher findings were reported by ***Ibrahim (2005)*** and ***Emam & El Nabrawy (2008)***. The present study illustrated that the heavy metals residues in milk samples from exposed buffaloes showed a significant increase when compared with those of control group and the acceptable limits recorcled by ***Carl (1991).*** Elevated metals residues in milk attributed to the positive correlation between both heavy metals in sources of environmental pollution (water, soil and feed stuffs) and those in blood of exposed buffaloes and consquently those in milk of those animals**.**

The heavy metals concentrations were determined based on plant dry weight (Table 4). ***Bailey (1977)*** recoded that the maximum tolerable level of Al for cattle and sheep is about 1.00 ppm.. Al concentration in plant sample (Darawa 2) exceeded the maximum tolerable level and the control values.

The maximum tolerable dietary level for Pb and Cd had been set at 20 mg/kg and 0.5 mg/kg for domestic animals respectively ***(Underwood & Suttle, 1999)***, also they recorded the normal level (1.6 mg/kg and 0.1-0.2mg/kg) of Pb and Cd respectively. The concentration of Cd in plant sample (Darawa 2) exceeded the maximum tolerable dietary level and the control values, while Pb value was within acceptable levels. The mean concentrations of Cu and Zn in the plant not excceded their maximum tolerable dietary level (25 mg/kg and 750 mg/kg) cited by ***Underwood and Suttle (1999)*** our results conicided with the finding of mineral concentrations of food recorded by ***Eman et al. (2008).***

It was concluded that the use of sewage waste water in agricultural lands enriched soils with heavy metals to concentrations that may pose potential environmental and health risk in the long term for both animal and human. So, it is recommended that the government planing should include sanitary protection of surface water against heavy metal pollution including hygienic disposal of agricultural discharges, industrial effluents and sewage.

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دراسة العناصر الثقيلة والتغيرات الهيموبيوكيمائية في دم وألبان جاموس تم تغذيته على دراوه خضراء مروية بمياه ملوثة بالصرف الصحي

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هدفت هذه الدراسة لتقييم تركيز بعض العناصر الثقيلة فى دم و ألبان الجاموس الحلوب الذى يتغذى على نباتات صيفية خضراء (الدراوه) مروية بمياه ملوثة بالصرف الصحى بجانب دراسة تأثير هذا التلوث على بعض مكونات الدم و السيرم. لذلك تم أخذ عينات دم من مجموعتين من الجاموس الحلوب (10 حيوانات لكل مجموعة) من قرية تل- محمد بمحافظة الشرقية. المجموعة الأولى تم تغذيتها على نبات الدراوه المروى بمياه نهر النيل (مجموعة ضابطة) والمجموعة الثانية تم تغذيتها على نبات الدراوه المروى بمياه ملوثة بالصرف الصحى (مجموعة معرضة). وأخذت أيضا عينات ألبان من المجموعتين بالإضافة إلى عينات من الدراوه التى تتغذى عليها هذه الحيوانات. وقد أوضحت النتائج ارتفاع تركيز كل من عناصر الألومنيوم، الرصاص، الكادميوم، النحاس و الزنك فى عينات الألبان الخاصة بالمجموعة المعرضة وأيضا فى نبات الدراوه المروى بمياه ملوثة. وبفحص الصوره الدمويه أشارت النتائج أن المعادن الثقيلة أدت إلى حدوث نقص معنوى فى العدد الكلى لكرات الدم الحمراء، تركيز الهيموجلوبين وحجم الخلايا المضغوطة بينما لم يحدث تغير معنوى فى العدد الكلى لكرات الدم البيضاء. وبتحليل السيرم لوحظ ارتفاع معنوى فى مستوى عناصر الألومنيوم، الرصاص، الكادميوم، اليوريا، والنشاط الانزيمى للأسبرتيت أمينو ترانسفيريزو الألانين أمينو ترانسفيريز وأيضا الفوسفاتيز القاعدى. بينما وجد نقص معنوى فى تركيز عناصر النحاس والزنك وأيضا الجلوكوز، البروتين الكلى والألبومين فى المجموعة المعرضة إذا ما قورنت بالمجموعة الضابطة. يتبين لنا من هذه الدراسة أن المعادن الثقيلة تتراكم فى دم و ألبان الحيوانات التى تتغذى على النباتات المروية بمياه غير ملوثة مما أدى إلى تغيرات كبيرة فى صورة الدم وبعض الوظائف البيوكيميائية.