

THE RELATION BETWEEN HEAVY METALS AND TRACE ELEMENTS LEVELS IN BLOOD OF SHEEP REARED ON SEWAGE-POLLUTED PLANTS

(With 3 Tables and One Figure)

By

EMAN EZZ-EL-DAWELA EL-SHARKAWY;
Y.A.EL-KATTAN* ; SAMERA A. SENOUSI**
and M.A. SALEH**

* Shebeen El-Kome, and ** El-Wadi El-Gadid laboratories,
Animal Health Research Institute
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العلاقة بين مستويات المعادن الثقيلة والعناصر النادرة في دم النعاج التي ترعى على النباتات الملوثة
بمياه الصرف الصحي

إيمان عز الدولة شرفاوي ، ياسر عبد العال متولي القطان ،
سميرة أحمد سنوسي ، مصطفى أحمد صالح

تزايد الاهتمام في السنوات القليلة الماضية لتقييم العلاقة بين التسمم بالعناصر الثقيلة ومشاكل التغذية. وقد هدفت هذه الدراسة لتقييم تركيز العناصر الثقيلة وعلاقتها بالعناصر الحيوية الأخرى في دم الأغنام التي ترعى على الحشائش المروية بمياه الصرف الصحي. بمنطقة عرب المدابغ بأسبوط ولذلك تم أخذ عينات دم من مجموعتين من النعاج (كل منها ٢٠ نعجة). كانت المجموعة الأولى ترعى على البرسيم المروي بمياه تهر النيل شرق مدينة أسبوط (مجموعة ضابطة) والمجموعة الثانية كانت ترعى على البرسيم المروي بمياه الصرف الصحي بمنطقة عرب المدابغ بمدينة أسبوط (مجموعة معرضة). وأخذت أيضا عينات من البرسيم الذي كانت ترعى عليه هذه النعاج. وقد تم تقدير تركيز كل من عناصر الرصاص والكاديوم والحديد والنحاس والزنك في عينات الدم والبرسيم. وقد أوضحت النتائج أن البرسيم المروي بمياه الصرف الصحي كان يحتوي على تركيز أعلى لكل من الرصاص (أكثر من ضعفين) والكاديوم (أكثر من ١١ ضعف) عن القيم الضابطة. بينما كان تركيز العناصر الحيوية مثل الحديد والنحاس والزنك لا يتعدى القيم المقبولة العليا الموصى بها في تغذية الأغنام. ومن ناحية أخرى كان دم النعاج المعرضة يحتوي على تركيز أعلى لكل من الرصاص (أكثر من ٤ أضعاف) والكاديوم (أكثر من ٨ أضعاف). وكانت البلازما في النعاج المعرضة تحتوي على تركيز أقل لكل من الحديد والنحاس والزنك بالمقارنة بالمجموعة الضابطة. وقد أوضح معامل ارتباط بيرسون وتحليل الانحدار الخطي أن تركيز الرصاص كان يرتبط سلبيا بدرجة معنوية مع تركيز الحديد. ومن ناحية أخرى كان هناك ارتباط سلبي معنوي بين تركيز الكاديوم وتركيز كل من الحديد والنحاس والزنك. وتخلص هذه الدراسة إلى أن عناصر الرصاص والكاديوم تتراكم في الحيوانات التي ترعى على الحشائش المروية بمياه الصرف الصحي بتركيز أعلى عن الحيوانات التي ترعى على الحشائش الموجودة في المناطق غير الملوثة. وأن التعرض إلى عناصر الرصاص والكاديوم له تأثير ضار على صورة العناصر الحيوية في الدم. وقد أوضحت الدراسة أيضا أن عنصر الرصاص له تأثير ضار أكثر من الكاديوم على حالة عنصر الحديد، بينما كان للكاديوم تأثيرا ضارا أكثر من عنصر الرصاص على التمثيل الغذائي لعنصري النحاس والزنك. وبذلك تكون هذه الدراسة قد أكدت على وجود علاقة بين التعرض البيئي للعناصر الثقيلة ومشاكل التغذية في الحيوان.

SUMMARY

In the past few years, increasing consideration has been given to evaluate the relation between heavy metal toxicities and nutritional problems. The aim of this study was to evaluate the heavy metal concentrations and their correlation with the other essential bio-elements in blood of sheep reared on sewage-irrigated pasture. Blood was sampled from two groups of ewes (n=20 each), the first reared on Barseem (*Trifolium alexandrinum*) grown in a rural area east of Assiut city, where irrigation was carried out by the River Nile water (controls) and the second reared on sewage-irrigated Barseem in Arab El-Madabegh region, in the north of Assiut city (exposed). Barseem allowed for these animals was also sampled. Concentrations of lead (Pb), cadmium (Cd), iron

(Fe), copper (Cu) and Zinc (Zn) were estimated in blood and food samples. The results showed that polluted foods contained higher concentrations of Pb (>2 fold) and Cd (>11 fold) than the control values. Concentrations of the biometals Fe, Cu and Zn in polluted and normal foods did not exceed the maximum tolerable level recommended for sheep nutrition. Blood of the exposed ewes had higher concentrations of Pb (>4 fold, $P > 0.001$) and Cd (>8 fold, $P > 0.001$) than the control values. Exposed ewes had lower plasma concentrations of Fe ($P = 0.002$), Cu ($p = 0.016$) and Zn ($P = 0.009$) compared with control values. Pearson's correlation and linear regression (R^2) analysis coefficient revealed that Pb concentrations were negatively correlated with Fe concentrations ($R^2 = 0.46$, $P < 0.001$). On the other hand, there was negative significant correlation between Cd concentrations and the concentrations of Fe ($R^2 = 0.22$, $P = 0.014$), Cu ($R^2 = 0.41$, $P = 0.002$) and Zn ($R^2 = 0.51$, $P = 0.0004$). In conclusion, animals reared on sewage-polluted pasture accumulate higher Pb and Cd than those reared on non-polluted areas. Furthermore, Pb and Cd exposure have hazardous influence on the essential minerals profile in the blood. Pb is more hazardous than Cd on Fe status, but Cd is more than Pb on Cu and Zn metabolism. This study emphasizes a relation between the environmental exposure to heavy metals and the nutritional problems occur in the exposed animals.

Key words: Lead, cadmium, trace elements sewage, sheep

INTRODUCTION

The pollution with sewage sludge represents a situation of potential animal exposure to naturally occurring environmental toxicities (Wilkinson *et al.*, 2003). Diverse industrial activities have also been implicated to contaminate the forages with subsequent elevated heavy metals in animals (Swarup *et al.*, 2005; Patra *et al.*, 2005). The animals allowed to graze on or fed with contaminated pastures or fodders are exposed to these pollutants leading to various health hazards (Thornton, 2002). Those animals serve as better indicators of the health impacts of toxic heavy metals than the toxicant-administered experimental individuals (Phillips *et al.*, 2005).

Heavy metals have always occupied a central place in the nature and represent an environmental hazard because once these metals enter the environment they cannot be destroyed (WHO, 2005). Therefore, it is critical to assess the distribution of these metals in the environment and also their health effects (Thornton, 2002). Pb and Cd are naturally occurring metals but have no biological nutritive function (He *et al.*, 2005). In terms of adverse effects on animal and human health, these metals are amongst the elements that have caused most concern and are considered toxic metals (McDowell, 2003).

In the past few years, increasing consideration has been given to interactions occurring in the organism between toxic metals and bioelements essential for life (WHO, 2005). In the risk assessment process, understanding the pathophysiological ways of the contaminant is a crucial step in addressing and reducing the likelihood toxicity (Peraza *et al.*, 1998). Nutritional studies indicate that nutritional deficiencies in a number of essential elements such as calcium, iron, zinc, copper and phosphorus may affect the toxicokinetic of heavy metals (ATSDR 2005). On the other hand, many experimental animal studies showed that supplementation with Ca, Zn, or Cu decreased the absorption, retention, and toxicity of heavy metals (Fergusson, 1990; Underwood and Suttle, 1999 and McDowell, 2003).

The toxicity of Pb and Cd may largely be explained by its interference with different enzyme systems by displacing other essential metal ions (WHO, 2002). Various toxic and essential metals can interact by influencing each other's absorption, retention, distribution, and bioavailability in the body (Nordberg, 1978). This is mainly because of their competition for the same binding site (Elsenhans *et al.*, 1991). The intensity of most metal interactions depends on the tissue pH and redox potential, the concentrations of metals and exposure duration (Goyer, 1995).

Although heavy metal toxicities were extensively studied under experimental conditions (Neathery and Miller, 1975; Stoev *et al.*, 2003), studies are less established in domestic livestock under practical conditions. The level of a metal in blood is considered as an index of biologically active metal in the body and reflects a current environmental exposure (McDowell, 2003). Determination of blood heavy metals is useful in cases with minimal accumulation of these metals in the tissues, so that estimation of metal elements in the blood has a significant value for animal health (Wittman and Hu 2002).

The aim of this study was to evaluate heavy metals (Pb and Cd) concentrations in blood and their correlation with the essential bio-elements (Fe, Cu and Zn) in plasma of sheep reared on sewage irrigated pasture in Assiut city.

MATERIALS and METHODS

Animals:

Two groups of Balady ewes (20 each), aged 4-5 years were used in this study. The first group (clinically healthy control) was selected from animals reared in a rural area east of Assiut city, where irrigation was carried out by the River Nile water. The second group (exposed) was selected from animals rearing on pastures irrigated with sewage wastes at Arab El-Madabegh region (in the north of Assiut city).

Sampling:

Random samples (about 0.5Kg each) of Barseem (*Trifolium alexandrinum*) were collected from grazing areas of the two localities (5 from each locality). Samples of each locality were pooled, air-dried, wet digested (AOAC, 1995) and stored until biochemical analysis. Blood samples, each of 10 ml, were drained from the jugular vein of control and exposed ewes, using K₂-EDTA as anticoagulant. Each blood sample was divided into two parts (5 ml each). The first part was used for separation of plasma and the second was wet digested with nitric and perchloric acid. Plasma and digested blood samples were stored at -20°C until analysis.

Metal analysis:

Pb and Cd concentrations in the digested samples of blood and foods were estimated using graphite furnace atomic absorption spectrophotometer (Perkin-Elmer 4300 AA). Concentrations of Fe, Cu and Zn in the plasma and foods were determined using flame atomic absorption technique (GBC 932 AA).

Statistical analysis:

Blood biochemical data were expressed as means \pm standard error (SE). Differences between groups were determined using an analysis of variance followed by the Student t-test. Pearson's correlation (r) and linear regression analysis (R²) were performed on paired data obtained by individual cases. Significance level was set at P \leq 0.05.

RESULTS

Concentrations of heavy and biometals in foods (Barseem) allowed for sheep in both normal and polluted areas are presented in table 1. Polluted foods contain higher concentrations of Pb (>2 fold) and Cd (>11 fold) than the corresponding values in non-polluted foods. Concentrations of biometals Fe, Cu and Zn in polluted and normal foods did not exceed the maximum tolerable level of these minerals cited by Underwood and Suttle (1999).

Table 2 shows the mean concentrations (\pm SE) of blood Pb and Cd and plasma Fe, Cu and Zn (μ mol/l) in normal and exposed ewes. Blood of exposed ewes had higher mean concentrations of Pb (>4 fold, P >0.001) and Cd (>8 folds, P>0.001) than the corresponding values in normal ewes. Exposed ewes had lower mean concentrations of the biometals Fe (P=0.002), Cu (p=0.016) and Zn (P=0.009) than the corresponding values of normal ewes.

Table 3 and Fig. 1 show the Pearson's moment correlation (r) and linear regression analysis (R²) of paired data obtained by individual cases between heavy metals (Pb and Cd)

concentrations and Biometals (Fe, Cu and Zn). Individual Pb concentrations were significantly correlated in a negative mode with the concentrations of Fe ($R^2=0.46$, $P<0.001$). Both Cu ($R^2=0.11$, $P=0.123$) and Zn ($R^2=0.13$, $P=0.08$) concentrations were not significantly correlated with Pb values. On the other hand, there was negative significant correlation between individual Cd concentrations and the concentrations of Fe ($R^2=0.22$, $P=0.014$), Cu ($R^2=0.41$, $P=0.002$) and Zn ($R^2=0.507$, $P=0.0004$).

Table 1: Metal concentrations (mg/kg DM) in Barseem (*Trifolium alexandrinum*) from normal and exposed areas.

	Estimated values		Dietary levels*	
	Normal	Exposed	Normal level	maximum tolerable level
Pb	4.2	18.1	1-6	20.0
Cd	0.17	1.9	0.1-0.2	0.50
Fe	319	406	>100	500
Cu	12.2	16.7	>8	25
Zn	32	51	>22	750

*Underwood and Suttle (1999).

Table 2: The mean concentrations (\pm SE) of blood Pb and Cd and plasma Fe, Cu and Zn (μ mol/l) in normal and exposed ewes.

	Normal	Exposed	P-value	Toxic level*
Pb	71.8 \pm 3.9	294.5 \pm 22.6	>0.001	>500.0
Cd	0.034 \pm 0.007	0.298 \pm 0.021	>0.001	>0.089
Fe	33.6 \pm 0.57	30.6 \pm 0.49	0.002	-
Cu	10.3 \pm 0.38	8.9 \pm 0.38	0.016	-
Zn	8.8 \pm 0.30	7.9 \pm 0.28	0.009	-

*Underwood and Suttle (1999).

Table 3: Correlation of blood Cd and Pb with other plasma bio-metals:

	Statistic criteria*	Fe	Cu	Zn
Pb	r	0.689	0.206	0.281
	R^2	0.46	0.11	0.13
	P	0.0008	0.123	0.081
Cd	r	0.515	0.640	0.712
	R^2	0.22	0.410	0.507
	P	0.014	0.0024	0.0004

*r: Pearson's correlation, R^2 , Linear regression, P: level of significance.

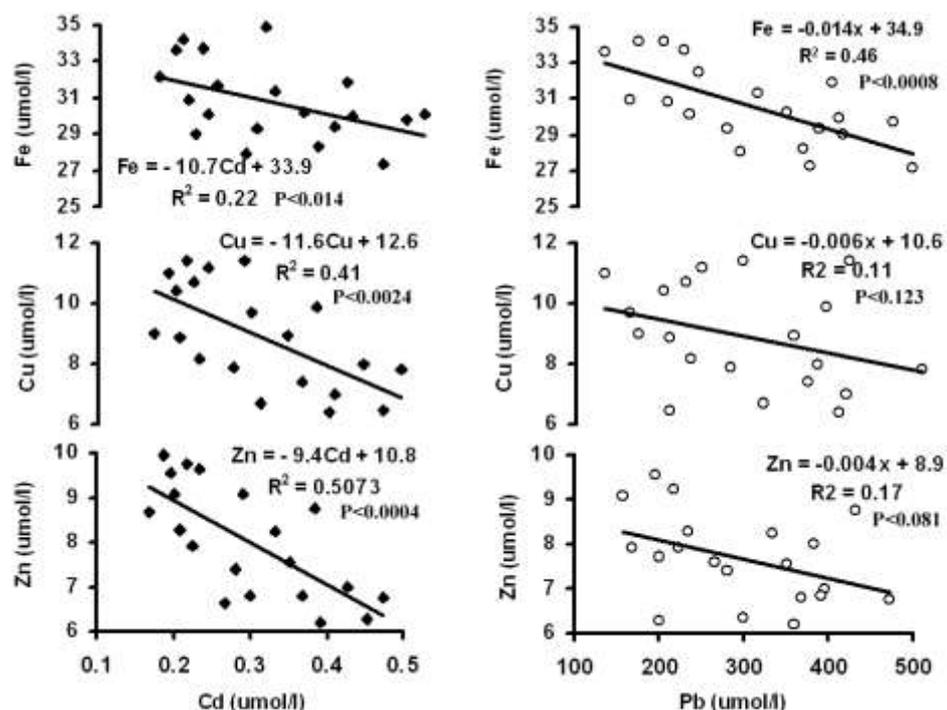


Fig. 1: The regression equation and regression factor (R^2) between heavy metals (Pb and Cd) and bio-metals (Fe, Cu and Zn).

DISCUSSION

The maximum tolerable dietary level for the toxic elements Cd and Pb had been set at 0.5 mg/kg and 20 mg/kg for domestic animals, respectively (NRC, 1980). The mean concentrations of Cd, but not Pb in the forage samples in the studied area largely exceeded the above reported data and control values. The mean concentrations of Fe, Cu and Zn in the forage not exceeded their maximum tolerable dietary level (500, 25 and 750 mg/kg) cited by NRC (1980) but they were higher than controls. Our results are in good agreement with the findings of mineral concentrations of food reported by Gomah (2001) in the same area.

The results of this study showed that ewes grazing on the sewage wastewater irrigated area were concentrating higher Pb and Cd in their blood than those grazing non-polluted area. As for many elements, metal levels in tissue are largely dependent on the metal content of diet (McDowell, 2003). Blood Pb and Cd levels broadly reflect amounts of these elements in the food (Thornton, 2002). Underwood and Suttle (1999) reported that the blood Pb level of 500 $\mu\text{mol/l}$ is the critical level of poisoning, but a value of 0.089 $\mu\text{mol/l}$ is the blood toxic level of Cd in sheep. Blood Pb concentration in the present study did not reach the toxic level of sheep, but Cd concentration exceeded the toxic values. This may depend on the fact that Pb ingested by sheep is poorly absorbed by the alimentary tract, and 99% is excreted in faeces (Nordberg, 1978). On the other hand, Phillips *et al.* (2005) suggested that Cd is absorbed more efficiently by sheep if it is in the organic form in grass. Retention of heavy metals in the total animal from sludge ingestion averaged 0.09% and 0.3% for Cd and Pb; no retention was noted from Cu and Zn (Johnson, *et al.* 1981). Studies of Wilkinson *et al.* (2003) showed that Cd was the only metal to accumulate consistently in increased toxic amounts in sheep allowed to graze pastures variously treated with sludge.

Understanding the pathogenesis of contaminants strengthens our ability to quantify or to predict the likelihood of effects (ATSDR, 2005). The toxicity of heavy metals have been widely studied and reported (Järup *et al.*, 1998 and ATSDR, 2005). These metals are environmental and occupational contaminant and may represent a serious health hazard to man and animals (Staessen *et al.*, 1999 and Prüss-Üstün *et al.*, 2003). The basis of Cd toxicity depend on its

negative influence on enzymatic systems of cells, resulting from substitution of other metal ions (mainly Zn^{2+} , Cu^{2+} and Ca^{2+}) in metalloenzymes (Jacobson and Turner, 1980; Stohs and Bagchi, 1995). Many effects of Cd action result from interactions with necessary micro- and macroelements, especially Ca, Zn, Cu, Fe and Se (Brzóska and Moniuszko-Jakoniuk, 1998; Peraza *et al.*, 1998). On the other hand, Pb poisoning inhibits the activities of SH-dependent enzymes, and interrupts heme synthesis at the level of formation of protoporphyrin and causes accumulation of delta-aminolevulinic acid (ATSDR, 2005). In the current work, in spite of the satisfactory essential elements in the diet, the mean concentrations of Fe, Cu and Zn in plasma of heavy metal exposed ewes were lower than the corresponding values in non-exposed individuals. It is suggested that the interaction of heavy metals with the essential metals in exposed group play a role in reduction of these bio-metals. Similar results were reported for human (Pizent *et al.*, 2003) and in cattle inhabits around different polluting sources (Patra *et al.*, 2005).

One of the symptoms associated with Pb and Cd intoxication is the development of anaemia in the exposed individuals (WHO, 2005). This could be attributable to the interference of Cd with iron absorption at the intestinal level (Stonard and Webb, 1976). Cd binds to liver ferritin, which is also present in the intestinal mucosa and involved in the mucosal uptake and transfer of iron (Vahter *et al.*, 1996). Likewise, Pb interferes with mitochondrial energy metabolism, which is necessary to reduce ferric iron to ferrous iron before insertion of iron into the porphyrin ring (Moore and Goldberg, 1985). High Pb and Cd concentrations in the blood decreased iron stores in children (Osman *et al.*, 1998). In the current study, a negative correlation was noticed between blood Pb and Cd concentration and Fe levels in plasma of exposed ewes. In this situation, Fe levels decrease when Pb and Cd values increase. Similar observation was noticed by Tripathi *et al.* (2001) who found good negative correlation between blood Pb and iron in human. These findings were believed to be due to competitive or antagonistic inhibition between these two elements (Vahter *et al.*, 1996). In the current study, the linear regression between Pb and Fe ($R^2=0.46$, $P>0.001$) was greater than that between Cd and Fe ($R^2=0.22$, $P=0.014$). These findings suggest that Pb had a more hazardous influence than Cd on Fe metabolism.

In the current work, plasma Cu was negatively correlated with blood Cd, and not correlated with blood Pb. This indicates that Cu concentrations decrease as Cd concentrations increase. Mills and Dalgarno (1972) found that copper metabolism was seriously disturbed in pregnant ewes receiving Cd in their diet. The explanation for the Cd-copper interaction is that the two metals compete for binding sites on metallothionein. Copper displaces Cd from metallothionein because of its higher affinity for the protein (Funk *et al.*, 1987).

Plasma Zn in exposed sheep was negatively correlated with blood Cd concentrations, and not correlated with blood Pb in the present study, suggesting a decreased Zn concentration as Cd levels increased. In biological systems, Cd and Zn are linked to macromolecules primarily through sulphur (S), oxygen (O) and nitrogen (N) and interact readily with S-, O- and N- donors. They bind preferentially to the same proteins as albumin in the bloodstream and metallothionein and other proteins in tissues (Brody, 1999). Cd^{2+} and Zn^{2+} ions can compete, and Cd may displace Zn in a number of biological processes (Endo *et al.*, 1997). Thus, Cd^{2+} interacts with Zn^{2+} at stage of absorption, distribution, accumulation and at the stage of excretion (Brzóska and Moniuszko-Jakoniuk, 2001).

In conclusion, animals reared on sewage-polluted pasture accumulate higher Pb and Cd than those reared on non-polluted areas. Furthermore, Pb and Cd exposure have hazardous influence on the essential minerals profile in the blood. Pb has a more hazardous influence than Cd on Fe metabolism, but Cd has a more deleterious effect than Pb on Cu and Zn metabolism. This study emphasizes the relation between environmental exposure to heavy metals and the nutritional problems occur in the exposed animals.

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