Relationship between soil cobalt and vitamin B_{12} levels in the liver of livestock in Saudi Arabia: role of competing elements in soils.

Etimad A. Huwait[1,2,3], Taha A. Kumosani[1,2,4], Said S. Moselhy[1,2,5,6], Rami M. Mosaoa[1], Soonham S.Yaghmoor[2,4]

1. Biochemistry Department, Faculty of Science King Abdulaziz University, Jeddah, Saudi Arabia.
2. Experimental Biochemistry Unit, King Fahd Medical Research Center. King Abdulaziz University.
3. Vitamin D Pharmacogenomics Research Group, King Abdulaziz University.
4. Productions of Bioproducts for Industrial Applications Research Group, King Abdulaziz University.
5. Bioactive Natural Products Research Group, King Abdulaziz University.
6. Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

Abstract

Objective: This study aimed to analyze the agricultural soils from different regions in Saudi Arabia for cobalt and related metals as Cu^{2+}, Ni^{2+},Cr^{3+}, Zn^{2+} and Pb^{2+}.

Materlais and methods: Liver and muscle tissues of livestock grazing on the selected areas were analyzed for the content of Co and vitamin B_{12}.

Results: Our results indicated that the levels of Co in surface soil (0-15 cm) were higher than in sub-surface soil (>15 cm-45 cm). In contrast, Pb and Zn were higher in sub-surface soil than in surface soil. A significant positive correlation existed between the levels of Co and vitamin B_{12} in the liver of livestock. However, Co was not detected in muscle tissues while vitamin B_{12} was present at very low levels in comparison with the levels found in the liver. The results indicated that Zn^{2+}, Pb^{2+} compete with Co in soil, which eventually affected the levels of vitamin B_{12} in liver.

Conclusion: It was recommended that survey of heavy metals in grazing fields of cattle should consider inclusion of multiple elements that compete with the bioavailability of essential elements in plants and animals for the prevention of deficiency of essential elements such as Co.

Keywords: Soil, vitamin B_{12}, cobalt, cattle, uptake, transfer

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Introduction

Many trace elements present in agro ecosystems including cobalt (Co) and selenium (Se) are not essential for plant growth but are required by animals and human beings. Cobalt is a core element of vitamin B_{12} (cyanocobalamin), which is essential for the prevention of anemia in humans[1]. Ruminant animals such as cows, sheep, goats, deer and camel produce vitamin B_{12} if there is adequate Co in their diet[2]. Three to 13 percent of the Co in the diet of a ruminant animal is incorporated by rumen bacteria into vitamin B_{12}. Although the liver of ruminants can store sufficient amounts of this vitamin for up to several months, vitamin B_{12} production in the rumen drops off rapidly within days, if there is a Co deficiency in the diet as well as a defect in absorption[3].

Corresponding author.
Said S Moselhy,
Biochemistry Department,
Faculty of Science,
King Abdulaziz University.
Email; moselhy6@hotmail.com
Tel:00966566991424.

Humans are totally dependent upon their intake of vitamin B_{12} from meat and livers of ruminant animals. Thus, ruminant animals play a vital role in human nutrition as a sole source of vitamin B_{12}. Areas that contain high or low concentrations of Co can have a direct relation to the health of plants, animals and humans in terms of vitamin B_{12} levels. There is a clear advantage in predicting the risk of Co deprivation in soil due to its significant biological value. Deficiency of soil Co would result in impairment in vitamin B_{12} formation in plants and animals and consequently pernicious anemia in humans[4]. Vitamin B_{12} is involved in the metabolism of proteins, phospholipids and neurotransmitters. Its deficiency leads to several neurological manifestations as fatigue, weakness, numbness, decreased memory, irritability, confusion and depression, although initial symptoms might often be vague. Vitamin B_{12} causing hyperhomocysteinemia and methylmalonic acidemia which can have serious health implications[5].

A condition in cattle and sheep known as "Phalaris staggers" results when these animals graze on Co-deficient soils. Animals will develop symptoms of muscular tremors, lack of coordination, rapid breathing and heart beat. Application of 4-5 ounces of cobalt sulfate, or supplementation of Co in the ration, can prevent this health complication[6]. Russian sheep grazing on Co-deficient pastures showed severe lung infection but when the soil was treated with Co, the incidence of this bacterial infection was greatly reduced[6]. Excessive amounts of antagonistic minerals can create shortfalls of Co uptake by plants and eventually, the ruminants[7]. Dietary Co supplementation in cows increased vitamin B_{12} levels in colostrum and milk. Furthermore, there was no effect of Co supplementation on dry matter intake or yield of milk[8]. Despite the significance of Co in soils on human health, very little is known on Co levels in soils in Saudi Arabia and the uptake of Co by ruminants through ingestion of plants and the role of antagonistic elements on Co uptake. The aim of the present work was to determine Co^{3+} and associated elements Cu^{2+}, Ni^{2+},Cr^{3+}, Zn^{2+}, Pb^{2+} in agricultural soils from different areas in Saudi Arabia including Taif, Jizan, Qassim, Hail and Tabuk. In addition livestock liver and muscle collected from animals grazing in these areas were analyzed for Co and vitamin B_{12} to evaluate the transfer of Co from soils to ruminants.

Materials and methods

Soil samples

Surface soil (0-15 cm) and sub-surface soil (>15-30 and 30-45 cm) samples were collected from 5 areas in the Kingdom of Saudi Arabia (Hail, Jizan, Qassim, Tabuk and Taif). Soils were collected with a stainless steel auger at 3 depths, 0–15 cm, 15–30 cm and 30–45 cm. Each sample was collected within a 1 m^2 area (4 corners and one central sub-sample) and combined to insure distribution of cobalt in soil. Soil samples were enclosed in sealable glass containers and stored at 4°C upon return to the laboratory. Portions of each soil sample were air-dried, at 60°C, gently ground with a pestle and mortar and passed through a 2 mm sieve.

Determination of heavy metals

One g of each soil sample was digested in 25 ml HNO3: HCl (1:3), 10 ml HF, 10 ml $HClO_4$ and heated overnight (200°C) until completely dried and then cooledat room temperature . Then, 20 ml of concentrated HCl was added and heated for 30 min. The volume was adjusted to 250 ml with deionized water. Cobalt level was determined using Atomic Absorption Spectroscopy[9].

For the analysis of Cu^{2+}, Ni^{2+},Cr^{3+}, Zn^{2+}, Pb^{2+} and Fe contents in soil, 0.1 g of milled, air-dried soil was digested in concentrated nitric and perchloric acids (4 ml HNO3:1ml HClO4) by heating at 195°C for 2 h. Two ml of 5 M HCl was added to the ash and heated at 60 °C for 1 h and then mixed with 8 ml deionized water, centrifuged for 20 min at 2000 rpm, and the solutions were then analyzed by Atomic Absorption Spectroscopy, Varian 1475 AA spectrophotometer with sensitivity 0.0001PPM[10].

Determination of liver cobalt and vitamin B_{12} levels

Liver and muscle samples from twenty camel were freshly collected after decapitation. Blood was removed with buffer saline and tranfered to ice container till delivery to the laboratory. Co levels in livestock liver and muscle were determined by atomic absorption spectroscopy as described earlier[12] and vitamin B_{12} levels in liver and muscle were determined by ELISA kit purchased from APLCO diagnostic (England) .The unknown concentration was calculated from standard curve[13].

Statistical analysis

The differences between surface soil and sub-surface soil heavy metal contents in various locations in Saudi Arabia were analyzed by ANOVA using SPSS program version 8.0.

Results

The concentrations of heavy metals including Co determined in soil samples from different regions of Saudi Arabia are shown in Tables 1 and 2.

Table (1):The mean concentrations of heavy metals (ppm, dry weight) in different soil samples at different levels in Jizan, Taif and Tabouk (mean± SD).

	Jizan			**Taif**			Tabouk		
Metal	**Surface soil**	**Sub-surface soil 15 cm**	**Sub-surface soil 30 cm**	**Topsoil**	**Subsoil 15 cm**	**Subsoil 30 cm**	**Topsoil**	**Subsoil 15 cm**	**Subsoil 30 cm**
Cobalt	4.1±0.5	2.6±0.2 [N.S]	1.2±0.03 [a]	2.1±0.5	1.3±0.2 [a]	1.4±0.03 [a]	4.1±0.5	2.6±0.2 [a]	1.2±0.03 [a]
Iron	2.5±0.06	2.3±0.2 [N.S]	1.9±0.5 [N.S]	3.3±0.9	3.9.3±33 [N.S]	2.9±5.5 [N.S]	2.8±0.06	3.3±0.3 [N.S]	3.9±0.5 [N.S]
Lead	1.19±0.01	2.5±1.6	3.3±0.2	1.19±0.01	2.5±1.6 [a]	3.3±4.2 [a]	1.19±0.01	2.5±0.6 [a]	3.3±4.2 [a]
Zinc	3.1±0.9	6.1±19.5 [a]	8.2±1.1	2.6±0.9	4.8±19.5 [a]	4.2±5.1 [a]	2.1±0.9	3.0±1 [a]	3.8±0.8 [a]
Copper	1.2±0.1	1.11±0.3 [N.S]	1.9±0.4 [N.S]	2.2±0.1	2.11±0.3 [N.S]	1.9±0.7 [N.S]	1.3±0.1	1.6±0.4 [N.S]	2±0.9 [N.S]
Nickel	0.9±0.01	1.0±0.02 [N.S]	1.8±0.3 [N.S]	0.9±0.01	1.0±0.02 [N.S]	1.1±0.4 [N.S]	1.1±0.01	1.13±0.02 [N.S]	1.9±0.3 [N.S]
Chromium	2.4±0.8	2.5±0.4 [N.S]	1.5±0.3 [N.S]	2.4±0.8	2.5±0.4 [N.S]	2.1±0.8 [N.S]	2.7±0.8	2.8±0.4 [N.S]	2.4±0.4 [N.S]

a: P< 0.05 was consider as significant
P value of subsoil (15 cm and 30 cm) versus topsoil.
N.S :Non significant.

Table (2): The mean levels of heavy metals (ppm) in different soil samples at different levels in Hail and Qassim (mean± SD).

	Hail			**Qassim**		
Metal	**Topsoil**	**Subsoil 15 cm**	**Subsoil 30 cm**	**Topsoil**	**Subsoil 15 cm**	**Subsoil 30 cm**
Cobalt	6.1±0.5	1.6±0.2 [a]	2.2±0.03 [a]	4.1±0.5	2.6±0.2 [a]	1.2±0.03 [a]
Iron	2.0±0.06	1.9±0.2 [N.S]	2.2±0.5 [N.S]	2.8±0.06	3.3±0.3 [N.S]	3.9±0.5 [N.S]
Lead	2.19±0.01	4.5±1.6 [a]	5.3±0.2 [a]	1.19±0.01	2.5±0.6 [a]	3.3±4.2 [a]
Zinc	3.1±0.9	6.1±19.5 [a]	8.2±1.1 [a]	2.1±0.9	4.0±1 [a]	4.8±0.8 [a]
Copper	2.2±0.1	2.11±0.3 [N.S]	2.9±0.4 [N.S]	1.3±0.1	1.6±0.4 [N.S]	2±0.9 [N.S]
Nickel	0.4±0.01	0.6±0.02 [N.S]	0.7±0.3 [N.S]	1.3±0.01	1.5±0.02 [N.S]	1.0±0.3 [N.S]
Chromium	2.4±0.8	2.5±0.4 [N.S]	1.5±0.3 [N.S]	2.1±0.8	2.3±0.4 [N.S]	2.4±0.4 [N.S]

a: P< 0.05 was consider as significant
P value of subsoil (15 cm and 30 cm) versus topsoil.
N.S: Non-significant.

The concentration of cobalt in surface soils was higher than in sub-surface soil (>15 cm to up to 40 cm). In contrast, lead and zinc were higher in sub-surface soil than in surface soil. Nevertheless, no significant difference in the levels of iron, copper, nickel and chromium were found between surface and sub-surface soils.
It was found that, the concentrations of cobalt in soils from Taif were higher than those from Jizan and Tabouk. Concentrations of vitamin B_{12} in the livers of livestock were the highest in the order of Tabouk> Taif > Jizan> Qassim> Hail whereas liver cobalt levels in livestock were in the order of Tabouk> Qassim > Taif > Hail > Jizan. There was a significant positive correlation between the levels of cobalt and vitamin B_{12} in the liver ($r=0.55$; $p<0.05$). In muscle, vitamin B_{12} levels were lower than those in liver while cobalt was not detected in muscle of livestock due to its accumulation in liver.

Table (3) .The mean Concentrations cobalt (ppm, wet or dry weight) and vitamin B_{12} (ug/Kg, wet or dry weight) in liver and muscle of livestock from different regions of Saudi Arabia (mean ± SD).

Tissue type	**Jizan**	**Hail**	**Taif**	**Tabouk**	**Qassim**
Liver B_{12}	309±33	290±29	315±39	340±31	307±24
Liver cobalt	23±5	29±6.4	37±7.2	40±10	39±11
Muscle B_{12}	11±2	10±3.2	7±1.5	12±4.2	15±3.3
Muscle cobalt	ND	ND	ND	ND	ND

ND: not detected

Discussion
Cobalt belongs to the family of trace nutritional elements that contribute to the survival of soil organisms, plant performance and health of animals. Cobalt level was determined in the blood of randomly selected individuals of different ages and gender from Saudi Arabia and found that some food items lack cobalt, indicating that a poor source of this metal .

In this study, cobalt concentrations in surface soil were higher than in sub-surface soil whereas, lead and zinc concentrations were higher in sub-surface soil than in surface soil. Toxic heavy metals can persist in the environment long enough and can be taken up by plants thus, reaching the food chain, and posing a threat to soil quality and biodiversity. Soil pollution with heavy metals is common in areas where sewage sludge is applied[15]. Some metals such as Zn and Cu are essential for the growth of organisms where they are trace element sfor metalloenzymes such as superoxide dismutace and cytochrome oxidase, while others (e.g. Cd and Pb) are toxic with no known beneficial functions[14]. Cadmium has been one of the most toxic metals that compete and interact with other essential metals such as Cu, Zn and Fe, and induce deficiencies of these metals in organisms[15]. Cadmium (cd) exhibits most of its toxicological properties from its chemical similarity to Zn, by replacing Zn in most metallo-enzymes especially those containing sulphydryl groups. You have to compare metal levels at least toxic metal levels in your study with other studies from other countries, for example compare Cd and Pb levels in Saudi soils with other reported values from other countries and mention high in Saudi or low? Also compare Co levels. Try to correlate soil Co and livestock Co in liver and write something about it.
There was a significant positive correlation between the concentrations of cobalt and vitamin B_{12} in the liver of livestock ($r=0.55$, $p<0.001$). However, cobalt was not detected in the muscle tissues and vitamin B_{12} was present at very low level in muscle in comparison with liver vitamin B_{12} level.

Two indicators are generally used to determine the cobalt status in animals. First, the plasma vitamin B_{12} level and the second, the level of the body stores of vitamin B_{12}, which is concentrated at 60% in the liver[17]. These two indicators are poorly correlated and are therefore complementary to each other. Hepatic vitamin B_{12} level was known to respond quadratically to dietary cobalt exposure[18]. Indeed, in cattle, hepatic vitamin B_{12} levels increased with the ingestion of cobalt to 205-236 µg/ kg-[1] dry matter. Vitamin B_{12} content in the liver clearly increased with the cobalt level in the whole diet of bovine[18].

Heavy metals in agricultural soils mainly arise from pesticide and fertilizer use as well as sewage sludge application, in addition to natural occurrence. These metals are accumulated in soil, taken up by plants and other organisms (bio-accumulation). Elevated accumulation can lead harmful levels while uptake by plants leads to the transfer of metals in the food chain to animals and humans[20].

The fate and bioavailability of heavy metals in the soil is controlled by a complex set of chemical reactions and by a number of physical and biological processes acting within the soil as well as other factors such as soil pH, organic matter, Fe-, Mn- and Al-oxides, clay content. Plant species, cultivars, and age also influence metal phytoavailability together with other environmental factors such as climatic conditions, management practices, irrigation water, soil salinity and topography[21]

In summary, levels of Zn^{2+}, Pb^{2+} compete with the level of Co in soil that affect the level of vitamin B_{12} in liver of livestock . The mechanisms for competition of Zn and Pb with Co uptake. Further studies are needed to determine heavy metals in grazing regions in Saudi Arabia to avoid anemia due to the deficiency of Co in animals.

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