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ESTIMATION OF SOME HEAVY METALS IN MEAT, LIVER AND KIDNEYS OF SHEEP DRINKING WATER POLLUTED WITH SEWAGE IN SOME VILLAGES OF ASSIUT GOVERNORATE

(With 3 Tables)

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تقدير مستويات بعض العناصر الثقيلة فى لحوم وكبد وكلى الأغنام التى تشرب مياه ملوثة بالصرف الصحى فى بعض قرى محافظة أسيوط

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أجريــت هذه الدراسة لتقدير معدلات العناصر الثقيلة ومدى تواجدها فى مياه بعض القنوات المائية التى تلقى فيها مياه الصرف الصحى ومخلفاته فى بعض قرى محافظة أسيوط (الدوير ــ البربا ــ الشناينة ــ كوم اسفحت ــ بنى فيز). كما شملت الدراسة فحص لحوم وكبد وكلى بعض الأغنام التى تشرب من هذه المياه الملوثة لتقدير معدلات العناصر الثقيلة فيها لما تتميز بــه هــذه العناصر من أثر تراكمى مما يؤدى إلى احتمال وصول بعض هذه العناصر إلى الإنســان عــن طــريق السلسلة الغذائية. لذا فقد تم أخذ عدد ٢٠ عينة من المياه الملوثة تم اختيارها من عدة قنوات مائية ملوثة بمياه ومخلفات الصرف الصحى فى بعض قرى محافظة أسـيوط. كمــا تــم اختــيار عدد ١٠ عينات من مياه الصنابير العذبة النقية فى نفس القرى استخدمت كمجموعة ضابطة. كما تم جمع عينات من ذبائح الأغنام التي تتراوح أعمارها ما بين ٣ إلى ٥ سنوات ، والتى ذبحت بمجازر القرى المختلفة بمحافظة أسيوط بعدد ٣٥ عينة من كل من العضلات، الكبد والكلى من الأغنام التى سبق أن شربت بصفة مستمرة من المياه الملوثــة بالصــرف الصــحى، وكذلك تم جمع عدد ١٠ عينات من كل من العضلات، الكبد والكلــى من ذبائح الأغنام والتى سبق شربها من مياه الصنابير العذبة فى نفس القرى والتى اســتخدمت كمجموعــة ضــابطة. ولقد تم إعداد وتجهيز العينات وتحليلها كيميائياً فى جهاز الإمتصــاص الــذرى لتقدير مستويات الرصاص والكادميوم والنحاس والزنك بها. هذا وقد أظهرت نتائج التحليل الكيميائى للمياه الملوثة بالصرف الصحى تواجد الرصاص والكادميوم والــنحاس والــزنك بتركيزات عاليه عن مثيلاتها فى مياه الصنابير العذبة وبمناقشة النتائج أتضــح أن متوسطات مستويات الرصاص والكادميوم والنحاس والزنك فى المياه الملوثة قد تجــاوزت الحــدود المســموح بهــا دوليا. وقد تبين أيضا من نتائج التحليل زيادة معدلات الرصــاص والكادميوم والنحاس والزنك فى لحوم وكبد وكلى الأغنام التى تشرب من المياه الملوثــة. وهــذه الزيادة معنوية جداً عند مقارنتها بمثيلاتها فى الأغنام التى تشرب من مياه

الصنابير. وبمناقشة النتائج أتضح أن جميع عينات عضلات وكبد وكلى الأغنام التى تشرب من المياه الملوثة قد تجاوزت الحدود المسموح بها من قبل الهيئات الدولية لعنصرى الرصاص والكادميوم ــ الأمر الذى يمثل خطورة على مستهلكى هذه اللحوم لما لها من أثر تراكمى فى جسم الإنسان. أما بالنسبة لعنصر النحاس فقد دلت نتائج التحليل على أن متوسط تركيز النحاس فى عضلات كبد وكلى الأغنام التى تشرب مياه ملوثة قد تعدى الحدود المسموح بها دولياً. أما عن تركيزات عنصر الزنك فقد أظهرت التحاليل أن تركيز هذا العنصر فى عضلات وكبد وكلى الأغنام التى تشرب من المياه الملوثة قد تعدى الحدود المسموح بها دوليا. ولقد ناقشت الدراسة المخاطر الصحية لهذه العناصر على مستهلكى هذه اللحوم وكذلك الاحتياطات اللازمة والحلول المناسبة لتجنب هذه المخاطر أو على الأقل الحد منها.

SUMMARY

The present work was planned to study the pollution of water with heavy metals in some drainage canals in which the sewage waste water was directly discharged at certain villages of Assiut Governorate. In addition, the study was planned to detect the residues of heavy metals in tissues and organs of sheep drinking from this polluted water. 20 water samples were collected from polluted canals and 10 water samples were collected from fresh tap water. In addition, samples were collected from carcasses of sheep (aging ranged from 4 to 5 years) with number of 35 of muscles, livers and kidneys from sheep previously drinking polluted water with sewage and 10 samples of muscles, livers and kidney were collected from carcasses of sheep previously drinking fresh tap water from the same villages and used as a control. All tissue samples were collected from different slaughter houses in certain villages and cities at Assiut Governorate. Samples were prepared and analyzed for determination of lead, cadmium, copper and zinc concentrations by using Atomic Absorption Spectrophotometer. The obtained results revealed that the presence of lead, cadmium, copper and zinc in water of canals polluted with sewage were highly significant elevated than those in tap water and higher in its concentrations than the international permissible limits. Concerning the levels of lead, cadmium, copper and zinc in muscles, kidneys and livers of sheep drinking polluted water were highly elevated than those of sheep drinking tap water. The highest concentrations of lead and cadmium were detected in all examined tissues and organs of sheep drinking polluted water, while the highest concentrations of copper were detected in muscles, liver, then kidneys of sheep drinking polluted water, which were also higher than the international permissible limits. Also the values of zinc in all examined tissue samples of sheep drinks polluted water were exceeded the permissible international limits. It was evident from these results that there was a correlation between accumulation of heavy metals in tissues of sheep and its concentration in water. Public health importance and the hazardous toxic effects of these heavy metals, as well as the suggestive recommendations to minimize pollution with these heavy metals were discussed.

Key words: *Water pollution, sewage waste water, heavy metals, meat and organs, sheep*

INTRODUCTION

Water pollution is a very critical environmental problem facing public health officials. The greatest volume of waste discharged the watercourse is sewage. Without treatment, crude sewage flows into natural water and causes pollution. Sewage contains debris wastes, sanitary products, faecal matter, washings, fats, water from domestic baths and anything washed down drain or flushed down the toilet (Dean and Suess, 1985; El-Marazky, 1988 and Omer *et al.,* 2004).

Chemical analysis of water polluted with sewage revealed a highly significant increase in heavy metals especially lead, cadmium, zinc and copper and increase in other trace elements (Fahim *et al.* 1995a and Radwan and Ali, 2003).

Heavy metals represent chemical residues which have a major role in animal and human health. These elements are cumulative poisons causing health injury through progressive and irriversible accumulation in the body as a result of ingestion of repeated small amounts (Alberti and Fidanz, 2002).

In relation to the hazard health effect of heavy metals under investigation:

Lead: it is recognized as a toxic metal which accumulate in the body due to its low rate of elimination. Chronic lead poisoning characterized by liver dysfunction, anemia, muscular pain, lead nephropathy and neuropathy of both central and peripheral nervous system (Goldfrank *et al.,* 1990). Lead has serious long term effects especially on children (Baghurst *et al.,* 1992). Pregnant mothers should take extracaution to avoid lead exposure because up to 50% of child's blood lead level can be absorbed through the mother's body (Zadorozhnaja *et al.,* 2000). Children which are exposed to lead poisoning produces many complications like speech delay, hyperactivity, attention deficit disorders, learning disabilities, behaviour disorders, hearing loss, mental retardation, lowered intelligent quotient and poor performance in school (Agency for Toxic Substance Disease Registry,1988 and Zadorozhnaja *et al.*, 2000). Lead affect reproduction in men and women. In men it affect on male gonads resulting in abnormalities in sperms, decreased sexual drive, unpotence and sterility. In women, it is associated with abnormal ovarian cycles and menstrual disorders in addition to spontaneous abortion, stillbirth and fetal macrocephaly (Needleman *et al.*, 1984). Potential carcinogenic nature of lead poisoning has also been reported by Zawurska and Medras (1988).

Cadmium: it is also a toxic metal can cause a broad spectrum toxicological and biochemical dysfunctions (Funakoshi *et al.*, 1995). Cadmium accumulates with the age in body tissues and causes renal failure (Friberg, 1984). Cadmium has a significant role in the incidence of many other diseases such as diabetes mellitus and hypertension (Merali and Singhal.,1977 and Nishiyama *et al.*, 1986). It is also a potent and effective plumonary carcinogen (Waalks and Rehm,1994). Cadmium is a major contributor to thyroid diseases (Watanabe *et al.*, 2000)..

Copper: it is an essential element for human and animals and acts as a cofactor for several enzymes. Copper is important in formation of erythrocytes, development of bone, central nervous system and counective tissues. Acute exposure to copper causes hypotension, haemolytic anemia and cardiovascular collapse, while chronic exposure resulted in jaundice in human (Gossel and Bricker, 1990).

Zinc: it is necessary for normal growth and development in animals and birds. Oral toxication by zinc leads to bloody watery diarrhea, intense abdominal pain, central nervous system depression and tremors (Casarett and Doull 1996).

Heavy metals cannot be destroyed or broken over long time of heat treatment or environmental degradiation. They are conservative or persistant type of pollutants and translocated through food chains to man and animals (Mahaffey, 1977 and Huang *et al.*, 2003).

The object of the present study is to determine the levels of heavy metals especially lead, cadmium, copper and zinc in the water polluted with sewage. Estimation of these metals in meat, liver and kidneys of sheep drinking from this polluted water in Assiut Governorate was also evidenced.

MATERIAL and METHODS

Samples and adopted methods :

1. Water samples : 20 water samples were collected from five water canals polluted with sewage waste water (four samples from each canal) nearby and at some distances from the site of sewage waste water disposal. Ten samples of water were collected from tap water at the same villages (El-Dewier, El-Berbaa, El-Shanina, Kom-Esfaht and Bany Faaz) and used as control.

The water samples were collected in clean glass bottles for chemical analysis according to APHA (1995) and Kegley *et al.*, (1998). Quantitative determination of lead, cadmium, copper and zinc were carried out by using Atomic Absorption Spectrophotometer (Perkin Elmer 2380 U.S.A).

2. Samples of diaphragm muscle, kidneys and liver were obtained from all sheep under investigation. A total of 105 samples (35 each of muscles, kidneys and liver) were collected from 35 adult sheep (3-5 years old) drinking polluted water and 30 samples (10 each of muscles, kidneys and liver) were collected from 10 adult sheep (3-5 years old) drinking fresh tap water and used as control. The samples were collected and put individually in clean acid washed polyethylene bags, labeled and kept in deep freezer at –20°C till digestion and analysis were taken place. All collected samples were obtained from different slaughter houses in some cites and villages at Assiut Governorate .

The collected samples were prepared and digested according to AOAC (1984); Richard and Rubinshapiro (1986) and Khan *et al.* (1995). Chemical analysis were carried out in all samples to determine the concentrations of lead, cadmium, copper and zinc by Atomic Absorption spectrophotometer (Prkin Elmer 2380 U.S.A). The estimated levels of these metals in each examined sample were recorded as ppm (mg/kg) wet weight.

Statistical analysis :

The obtained data were statistically analyzed using Software Computer Program (Spsswin, 1995).

RESULTS

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

Table 1: Concentrations (p.p.m) of heavy metals in fresh tap and polluted canal water:

Examined metals	Fresh tap water $\bar{X} \pm$ S.E	Polluted canal water $\bar{X} \pm$ S.E	Permiss limits of metals in water ppm (WHO,1984)
Lead (pb)	0.271 ± 0.048	1.873 ± 0.230**	0.05
Cadmium (cd)	0.014 ± 0.002	0.553 ± 0.013**	0.005
Copper (Cu)	0.460 ± 0.016	4.980 ± 0.329**	1.000
Zinc (Zn)	1.723 ± 0.230	8.366 ± 0.556**	5.000

The obtained results were mean $\bar{X} \pm$ standard error (S.E)
** Significantly different from control at P<0.01.

Table 2: Concentrations* (p.p.m) of heavy metals in muscles, liver and kidneys of sheep drinking tap water and sheep drinking polluted canal water:

Metals	Examined samples	$\bar{X} \pm$ S.E in sheep drinking tap water	$\bar{X} \pm$ S.E in sheep drinking polluted water
Lead (pb)	Muscles	0.396 ± 0.035	4.893 ± 0.622**
	Liver	0.719 ± 0.052	6.480 ± 0.809**
	Kidneys	0.534 ± 0.043	5.355 ± 0.485**
Cadmium (cd)	Muscles	0.047 ± 0.004	0.583 ± 0.072**
	Liver	0.089 ± 0.006	0.836 ± 0.091**
	Kidneys	0.072 ± 0.007	0.697 ± 0.083**
Copper (cu)	Muscles	1.758 ± 0.216	8.933 ± 0.889**
	Liver	3.633 ± 0.422	97.655 ± 7.403**
	Kidneys	2.214 ± 0.276	41.873 ± 5.623**
Zinc (zn)	Muscles	6.399 ± 0.728	88.920 ± 9.855**
	Liver	18.422 ± 1.345	74.533 ± 6.963**
	Kidneys	14.786 ± 1.891	63.788 ± 7.922**

P.P.m. = part per million = mg/kg
$\bar{X}$ = Mean values.
± (S.E) = standard error
** Significantly different from control at P<0.01.
* Wet Weight

Table 3: Recommend levels of lead cadmium, copper and zinc in food:

Metal	Heavy metals residues mg/kg (ppm) in examined samples of sheep drinking tap water		Heavy metals residues mg/kg(ppm) in examined samples of sheep drinking polluted water		FAO/WHO (1989) Permissible limit of some heavy metals in food
	Ex.samples	X :mg/kg (pp.m)	Ex. samples	X :mg/kg (pp.m)	
Lead (pb)	Muscles	0.396	Muscles	4.893	1 ppm
	Liver	0.719	Liver	6.480	
	Kidneys	0.534	Kidneys	5.355	
Cadmium (cd)	Muscles	0.047	Muscles	0.583	Not exceed 0.04 - 0.05 ppm
	Liver	0.089	Liver	0.836	
	Kidneys	0.072	Kidneys	0.697	
Copper (cu)	Muscles	1.758	Muscles	8.933	3.5 ppm
	Liver	3.633	Liver	97.657	
	Kidneys	2.214	Kidneys	41.873	
Zinc (Zn)	Muscles	6.399	Muscles	88.920	40 ppm
	Liver	18.422	Liver	74.533	
	Kidneys	14.786	Kidneys	63.788	

FAO: Food and agriculture organization
WHO: World Health Organization

$\bar{X}$: mean value ppm = mg/kg

DISCUSSION

1. Heavy metals in drinking water of sheep :

Table (1) revealed that the mean concentration (ppm) of lead in fresh tap and polluted canal water were 0.271 and 1.873, respectively. Although these concentrations were exceeded the permissible limits (0.05ppm) recommended by WHO (1984), yet, lead concentration in polluted canal water was considered highly significant increase than those of fresh tap water. Nearly similar findings were obtained by Abdel Nasser *et al.*, (1996). Moreover, Manal Sayed (1995) recorded that lead concentration in water from different areas of River Nile in Assiut Governorate was ranged from 0.314-1.940 ppm in winter and 0.200-1.300 ppm in summer. Lower lead values were stated by Shawkey *et al.* (1994) and Fahim *et al.*, (1995b). On the other hand, high levels of lead were detected by Tork (1989).

Concerning cadmium level in the examined water samples, the mean concentration values in fresh tap water (0.014 ppm) and polluted canal water (0.0553 ppm) were higher than the permissible limits obtained by WHO (1984). The result recorded in table (1) pointed that the mean concentration of value of cadmium in polluted canal was highly significant elevated than the fresh tap water. Similar observations were recorded in water samples collected from Giza-Egypt by Youssef and Haleem (1999).

The mean concentration of copper in the examined polluted water samples was 4.980ppm. this value considered highly significantly elevated than that obtained from tap water (0.460 ppm). Also it was higher than the level recommended by WHO (1984) which is 1 ppm. Nearly similar results were obtained by Tork (1989) and Fahim *et al.*, (1995a). Lower copper levels in water were stated by Daoud *et al.* (1999).

Analysis of water samples in examined canals showed that the mean value of zinc residue was 8.366ppm. This mean values was highly significantly elevated than that obtained from tap water (1.723 ppm). Also, it was higher than the level recommended by WHO (1984) which is 5.00ppm. Sewage is an important source for zinc, where man excretes between 7-20 mg zinc per day and could possibly contaminate water effluents (Frostner and Prosi,1979). It is concluded that the water of canals from which the sheep drinking was highly polluted with lead, cadmium, copper and zinc. The metals reach the water directly from sewage waste water which discharged in these irrigated canals. The presence of such elevated heavy metals in drinking water in turn may reflect bioaccumulation of these metals in sheep meat and organs.

2. Heavy metals is muscles, liver and kidneys of sheep :

Table (2) revealed that the mean concentrations (ppm) of lead in muscles, liver and kidneys of sheep drinking fresh tap water were 0.396, 0.719 and 0.534 respectively. Meanwhile, the mean concentrations (ppm) of lead in muscles, liver and kidneys of sheep drinking polluted canal water were 4.893 , 6.480 and 5.355 respectively. These results pointed that the mean concentration values of lead in all examined tissue samples of sheep drinking polluted water were highly significant elevated than those of sheep drinking fresh tap water. The permissible limit of lead in meat as recommended by FAO/WHO (1989) was 1 ppm, hence the obtained results revealed that all examined tissue samples of sheep drinking polluted water were exceeded the maximum permissible limit (table 3) . Such elevation of lead reflect a considerable pollution of water in investigated water canals. Thereby the recorded lead levels in muscles, liver and kidney of sheep drinking polluted water constitute health hazard to consumers .

Low lead levels in meat and organs of sheep were detected by Vos *et al.*, (1991); Boulis (1993). Mousa and Samaha (1993), Antoniou *et al.*, (1995); Attalla (1998); Daoud *et al.*, (1998) and Soliman (2002). Such variations of lead concentrations may be reffered to differences in age of animals, localities of animals, rearing of the animals as well as the differences in the degree of environmental pollution at which the sheep were fed or drink. This held the view reported by El-Sherif (1991) and Hafez (1995).

The mean concentrations (ppm) of cadmium levels in muscles, liver and kidneys of sheep drinking fresh tap water were 0.047, 0.089 and 0.072 respectively. Meanwhile the mean concentrations (ppm) of cadmium levels in muscles, liver and kidneys of sheep drinking polluted water were 0.583, 0.836 and 0.697 respectively. Relative high levels of cadmium were observed in liver and kidneys. Cadmium is a cumulative toxicant in the continental ecological cycling, it accumulated mostly in liver and kidney (Gracey and Collins, 1992). According to FAO/WHO (1989) the maximum limit of cadmium of food should not exceed 0.04-0.05 ppm. Thereby the recorded cadmium contents in examined tissues of sheep drinking polluted water in our study were exceeded this permissible limit (table 3). So the cadmium levels in all examined tissue samples of sheep drinking polluted water constitute hazard to consumers. Low cadmium levels in sheep tissues were reported by Boulis (1993); Mousa and Samaha (1993); Daoud *et al.*, (1998) and Omima Diab *et al.*, (2000). On other hand higher levels of cadmium were detected by Doyle and Pfander (1975); Youssef *et al.*, (1988) and Zmudzki and Szkoda (1995).

The high levels of cadmium in some other researchers may be due to the differences in the degree of environmental pollution which the animals were subjected to it and the degree of absorption of this metal. The actual extent of cadmium absorption depends on a number of dietary factors such as the intake of protein, calcium, vitamin D, zinc and other trace metals. Following absorption, cadmium is transported and bound to certain proteins of plasma and red blood cells to other sites throughout the body. This facts were confirmed by *Brozoska* and *Moniuszko* (1998) and *Jin et al.*, (1998).

The results illustrated in table (2) showed that the mean concentration (ppm) of copper in muscles, liver and kidneys of sheep drinking fresh tap water were 1.758, 3.633 and 2.214 respectively. While the mean concentrations (ppm) of copper in muscles, liver and kidneys of sheep drinking polluted water were 8.933, 97.655 and 41.873 respectively. These results pointed that the concentrations of copper in all examined tissue samples of sheep drinking polluted water were highly significant elevated than those drinking fresh tap water.

According to FAO/WHO (1989), the maximum permissible limit of copper was 3.5 ppm, hence, the obtained data revealed that the copper level in all examined tissue samples of sheep drinking polluted water higher than the permissible limit especially liver and kidneys (table 3). This high bioconcentration of copper in both organs may be attributed to the high movement of copper from different tissues to the liver for detoxification and then to kidney for subsequent excretion (Pounds, 1985). It may be also to the binding of heavy metals to metalothioncin enhances bioaccumulation in liver and kidney. This is supported by the results obtained by Bartik and Piskac (1981) which found that all copper content from food or water is stored firstly in liver then in kidney .

The analysis of the obtained data in table (2) showed that zinc concentrations (ppm) in muscles, liver and kidneys of sheep drinking fresh tap water were 6.399, 18.422 and 14.786 respectively. While the mean concentrations (ppm) of zinc in muscles, liver and kidneys of sheep drinking polluted water were 99.920, 74.533 and 63.788 respectively. These results pointed that zinc concentrations in all examined tissue samples of sheep drinking polluted water were highly significant increased than those drinking fresh tap water.

The permissible limit of zinc recommended FAO/WHO (1989) was 40 ppm, so the obtained results revealed that zinc concentrations in all examined tissue samples of sheep drinking polluted water were higher than this permissible limit (table 3). Nearly similar results were recorded by Youssef *et al.,* (1988). Lower levels of zinc in tissues of sheep were recorded by Boulis (1993).

CONCLUSION

From the present study, it could be concluded that heavy metal pollution problems in the studied water canals were serious as reflected by the high heavy metal concentrations recorded in the collected water samples. Also, heavy metals concentrations in examined tissue samples of sheep drinking from this polluted water were higher than the permissible limits, so consumption of such meat and organs constitute public health hazards through progressive accumulation of these metals inside the human body. Such obtained results reflected the importance of water chemistry in determining the bioaccumulation of the metals. Several million people allover the world are currently exposed to elevated concentrations of toxic metals and metalloids in the environment and suffering subclinical metal poisoning (Mirgu,1988).

Therefore the preventive measures intended for minimizing the pollution of water and meat with such metals are of significant concern, including:

1. Sanitary protection of water against heavy metals through governmental plane and periodical examination of water supplies should be done and assessed according to the international standards;
2. 2. Strict application of regulations on sewage waste water pollutants which discharged directly in the water canals and stringent controls should be taken to prevent the farmers and the owners of animals from drinking their animals from such polluted water.
3. Hygienic disposal of sewage must be applied to protect human health from dangerous effect of heavy metals.
4. Education of owners of sheep and the consumers about the risk of such pollutants should be applied through educational training programs.
5. To estimate human daily intake of chemical residue through food, total diet studies must be carried out in Assiut Governorate.

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