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HEAVY METAL ACCUMULATION IN ANIMAL TISSUES AND INTERNAL ORGANS OF PIGS CORRELATED WITH FEED HABITS

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Abstract

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The purpose of the study was to assess the risk to public health. This study investigates the presence of heavy metals (Cu, Zn, Cr, Pb, Cd and Ni) in the liver, kidney and muscle tissues of seven sows slaughter of indigenous black race from the region of Thessaly correlated with feed habits and the used feedstuff. The breeding was carried out in the experimental facilities of TEI of Thessaly where six species of different feed types were participated in equal amounts. The analysis of the samples was done by wet digestion and atomic absorption spectroscopy. The results compared with the maximum permissible (EFSA) shown that greater daily intake of Cu, Zn, Cr, Pb and Cd provided at 49%, 76%, 12%, 7% and 5% respectively of feed samples. The concentration of Ni in the feed ranged from 0.10 to 9.90 mg kg^{-1} (dm) dry matter. In muscle tissue, the concentration of Cu, Zn, Ni, Cr, Pb and Cd ranged from 0.30 to 1.23, 10.40 to 39.01, < 0.06, < 0.006, < 0.02 and < 0.02 mg kg^{-1} respectively (fm) fresh mass after fat removal. In the liver, concentration of Cu, Zn, Ni, Cr, Pb and Cd ranged from 2.71 to 5.94, 14.90 to 18.44, < 0.06 to 0.95, < 0.006 to 3.97, < 0.02 and < 0.02 mg kg^{-1} (fm) respectively. In kidney the concentration of Cu, Zn, Ni, Cr, Pb and Cd ranged from 2.44 to 7.02, 10.04 to 28.98, < 0.06 to 0.95, < 0.006 to 4.04, < 0.02 and < 0.02 to 0.14 mg kg^{-1} (fm) respectively.

Key words: Accumulation, conventional agriculture, heavy metals, pig feed

Introduction

Intensive animal production systems are on the increase in many regions of the world. Among them pigs are in great importance since is the main livestock in many countries (Gorni et al., 2010). However, increase animal production is not the only aspect in human feed. Food safety is also important. Thus, one of the major aspects of food safety is toxic substances such as heavy metals (Maas et al., 2011).

Nowadays, it is well known that damage to crops or livestock may be caused by trace metals present in, or added to soil (Wong, 1985). Soil is a long-term sink for heavy metals although; they have different mobility and bioavailability (Nicholson et al., 2006). Some metals may bio-accumulate in the food chain, causing human health and environmental concerns (Ihnat and Fernandes, 1996; Demirezen and Uruc, 2006; Toor et al., 2007). By nature, animal liver is a natural source of Fe and other essential elements, such as Cu, Mg, Zn and Mn. However, liver might contain higher amounts of heavy metals and other contaminants, which tend to accumulate in liver tissues (Adei and Forson-Adaboh, 2008).

Furthermore, heavy metals are not only found in soil and in water by human industrial activity but, according to Sage (2007) and Moral et al. (2008), are artificially added in commercial feeds which are often enriched with essential elements such as Cu, Zn, and As in order to promote optimum growth rate and to infuse antimicrobial properties.

Feeds may also contain other nonessential elements such as Cd, Pb, Cr due to their presence in concentrates and supplements and environmental pollution (McBride and Spiers, 2001; Li and Chen, 2005; Sage, 2007). Thus, heavy metal contents showed large variation among the feed samples, indicating the differences in the use of feed additives among farms (Wang et al., 2013). Reducing heavy metal inputs in agricultural soils is an important strategic aim to protect farmland and ensure food safety, thus EU develop the appropriate protection policies (EC, 2001; Moral et al., 2008).

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Studies have shown that animals raised in industrial areas have higher concentrations of heavy metals in their internal organs, than animals reared in rural areas (Abou-Arab, 2001). Similar studies in metal processing areas in eastern Kazakhstan, showed high concentrations in feed and greater accumulation of Cd, Pb, Zn in cattle compared to sheep (Farmer et al., 2000). Furthermore, in polluted areas with rust scrub metals in Nigeria where dairy cows were raised, concentration of Pb in blood, milk and animal wastes, increased significantly compared with cows raised in uncontaminated areas of the country (Ogundiran et al., 2012). Finally, studies on the distribution of heavy metals in liver, kidney, heart, pancreas and muscle tissues of various animals taken from Kohat Market Pakistan, showed higher concentrations in the liver and kidneys and lower in muscle tissues (EI- Salam, 2013).

The objectives of this study were to determine the heavy metal contents in several internal organs such as liver, kidney and muscle tissue of selected piglets in the region of Thessaly correlated with feed habits and the used feedstuff assessing the risk to public health.

Materials and Methods

The experiment was conducted from August 2011 until May 2012 at the Technological Educational Institute of Thessaly (T.E.I), in the experimental animal holdings. Experimental facilities were more than 4 km away from any industrial units and 1 km away from highway. Seven, 60 days old, sows slaughter of indigenous black race were purchased from domestic pig farm from the region of Pieria-Macedonia. The purchased piglets had excellent health and weight between 12-15 kg. The pig feed were collected from conventional commercial farms in the area of Larissa-Thessaly and were contained wheat, barley, maize, wheat straw, soya bean meal and fattening mixture. The participation in the diet of pigs of the above pig feed was equal. Concentrations of heavy metals (Cu, Zn, Ni, Cr, Pb and Cd) were analyzed periodically throughout the period of the experiment collecting 30 samples of each type of piglet with three repetitions. The used drinking water was also examined for heavy metal's concentration.

The feed amount was represented at the 5% of the live weight of the piglets. Health condition was observed by a veterinarian of T.E.I of Thessaly. The age of the slaughter pigs was 350 days old and the weight of them varies about 90 kg. The slaughter were took place at the municipal slaughterhouse of the city of Larissa. The use of animal was treated according to the National guide of care and use of laboratory animals.

The processing of meat for the determination of heavy metals was performed in the laboratory of agricultural chemistry on the same day of the slaughter. In this procedure, fat was removed from muscle tissues while kidneys and liver was used as fresh tissue mass. Twenty one samples (3 samples from each pig) with three replicates from each one, and 21 samples of the kidneys and the liver were then analyzed.

Determination of heavy metals

One g of feed dry matter, 50 ml of drinking water, 5 g of muscle tissues (after removal of fat), 5 g of fresh mass liver and kidneys after homogenization were analyzed by wet combustion (350^{0}C) in the presence of 10 ml of HNO_3 + 5 ml of $HCLO_4$ (Allen et al., 1974; Varian, 1989) and measured by Atomic Absorption Spectroscopy Varian Spectra AA 200 (Victoria, Australia). The Cu and Zn were determined by the flames and mix (air-acetylene), while Cd, Ni, Cr, and Pb were determined using graphite furnace. Fisher's test procedures were used to detect and separate the mean treatment differences at P = 0.05. ANOVA statistical analyses were performed by the statistical program MINITAB (Ryan et al., 2005).

Results and Discussion

From the results presented in Table 1 it was observed that Cd was detected in 8% of the total amount of samples (180 feed samples) with an average content of 1.72 ± 0.18 mg kg^{-1} (dm) dry matter. Moreover, Pb was detected in 21% of samples with an average content of 10.41 ± 1.62 mg kg^{-1} (dm) and Cr was detected in 22% of the samples with an average content of 9.47 ± 0.79 mg kg^{-1} (dm). According to European Food Safety Authority (2009), the maximum daily intake limits of Cd, Pb, Cr for full board in pigs are 1, 10 and 13.3 mg respectively. Comparing the results of the analyzes of feed with higher daily intake limits Cd, Pb, and Cr, for daily food 4 kg equal to 5% of body weight of pigs, at 5% of the feed Cd concentrations of samples corresponding to Cd uptake more the maximum daily Cd intake threshold for pigs. Representing 7% of the feed samples, the Pb concentrations corresponded to uptake of Pb higher than the maximum daily limit intake Pb, while the proportion of feed corresponding to higher daily intake of Cr was 12%. Also, from Figure 1 it was observed

Table 1
Heavy metal concentrations (mg kg^{-1} dry matter) in piglet's feedstuff

Cd	Pb	Cr
<0.02 *(166)*	<0.02 *(142)*	<0.06 *(140)*
1.72±0.18 *(14)*	10.41±1.62 *(38)*	9.47±0.79 *(40)*

Data represent average means and SE deviation. *(n)*: Number of sample that the average content of Zn in the feed used to feed piglets were 135.03 ± 9.25 mg kg^{-1} (dm), of Cu 67.38 ± 6.50 mg kg^{-1} (dm), and Ni 3.88 ± 0.26 mg kg^{-1} (dm). Higher daily Cu and Zn intake limits for complete feed (EFSA, 2009) for pigs is 170 and 150 mg respectively. Comparing the results of the concentrations of Cu and Zn in feed at the maximum daily intake levels for pigs 80 kg and daily feed 5% of body weight, 49% and 76% of the samples of the feed concentrations of Cu and Zn respectively, accounted for Cu and Zn uptake greater than the maximum daily intake limit. Since a number of feed samples found high rates of heavy metals, feed from conventional farms in the region of Thessaly should be improved.

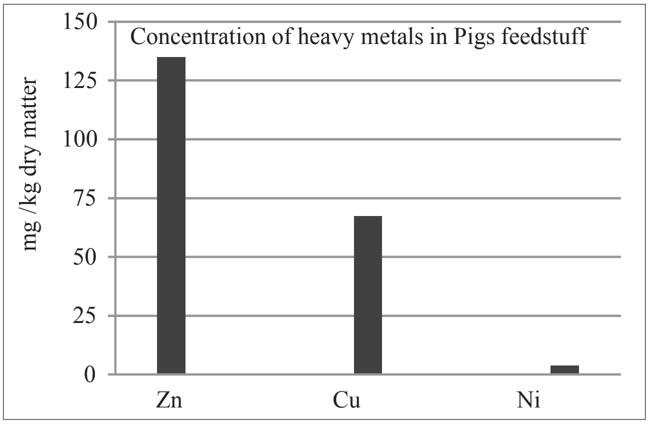

Fig. 1. Concentration of heavy metals: Zn, Cu and Ni in piglets feedstuff. Data represents average means and standard error (SE) N=180. Zn=135.03±9.25, Cu=67.38±6.50, Ni=3.88±0.26

The water used for drinking from the piglets was of good quality with low content of heavy metals. Results of water analysis (5 different samples during the experimental time) are shown in Table 2.

However, from the results shown in Table 3, on the accumulation of Cd, Cr, and Pb, in muscle tissue, kidney and liver of the examined pigs, it was observed that in 35% and 30% of kidney samples detected Cd and Cr in average concentration of 0.09 ± 0.02 mg kg^{-1} Cr and average concentration of 2.98 ± 0.43 mg kg^{-1} respectively. Moreover, Cr detected in 70% of the examined liver samples, with an average concentration of 2.01 ± 0.35 mg kg^{-1}. Unlike Pb were undetectable (< 0.02 mg kg^{-1}) in all examined samples of muscle tissue, kidney and liver.

Since a higher Cd levels in muscle tissue of pig, kidney and liver designated 0.050, 1.0, and 0.5 mg kg^{-1} fresh weight respectively, and Pb in pig meat designated 0.1 mg kg^{-1} fresh weight (EFSA, 2010; 2012), it was observed that in all samples (muscle tissue, kidneys and liver) measured concentrations of Cd and Pb were lower than the maximum permissible limits.

Due to the low concentrations of Cd, Pb, Cr, and Ni (< 0.02, < 0.02, < 0.006 and < 0.06 mg kg^{-1}, respectively) found in muscle tissues of pigs and since the maximum daily limit intake Cd, Pb, Cr, and Ni for an adult, is 70 µg, 250 µg, 250 µg, 300 µg respectively (Codex Alimentarius Commission, 2001; Commission Regulation, 2006), the consumption of muscle tissue of pigs from the region of Thessaly has no restriction as to the concentrations of these metals.

Table 2

Heavy metal concentrations (mg L^{-1}) in used drinking water

Cu	Zn	Ni	Cd	Cr	Pb
0.15±0.02	2.23±0.08	0.0155±0.001	0.0029±0.001	0.0291±0.003	0.0068±0.002

Data represent average means and SE deviation for 5 water samples

Table 3

Heavy metal concentrations (mg kg^{-1} of fresh tissue) in liver, kidney and muscular tissues

Internal organs of pigs	Cd	Pb	Cr	Ni
liver	<0.02 *(21)*	<0.02 *(21)*	<0.006 *(6)*	<0.06 *(15)*
			2.01±0.35 *(15)*	0.63±0.14 *(6)*
kidney	<0.02 *(15)*	<0.02 *(21)*	<0.006 *(15)*	<0.06 *(18)*
	0.09±0.02 *(6)*		2.98±0.43 *(6)*	0.84±0.11 *(3)*
muscular tissues	<0.02 *(21)*	<0.02 *(21)*	<0.006 *(21)*	<0.06 *(21)*

Data represent average means and SE deviation. *(n)*: Number of samples

In contrast, comparing the higher concentrations of Cd, Pb, Cr, and Ni (< 0.02, < 0.02, 3.97 and 0.95 mg kg^{-1} respectively) found in the liver of pigs, to the corresponding maximum daily intake limits, we conclude that 63 g daily by liver fever is enough to fill the maximum daily intake limit Cr for adult (Table 3).

However, compared the largest concentrations of Cd, Pb, Cr, and Ni (< 0.02, < 0.02, 4.04 and 0.95 mg kg^{-1} respectively) found in the kidneys of the pig, with the respective maximum daily intake limits, it was observed that 61.9 g per day from the kidneys of pigs is enough to reach the maximum daily intake limit of Cr to an adult. Results in lower concentrations of heavy metals in muscle tissue relative to the internal organs of the pigs have been found by other authors (EI-Salam, 2013).

Furthermore, low levels of Ni were observed in 30% of the samples of liver, 15% of samples of kidney and not at all in sample of muscle tissue.

The concentration of Cu in muscular tissues, liver and kidney of pigs ranged from 0.3 to 1.23, 2.71 to 5.94 and from 2.44 to 7.02 mg kg^{-1} (fm) respectively. However, the concentration of Zn in muscular tissues, liver and kidney of pigs ranged from 10.40 to 39.01, 14.90 to 18.44, and 10.04 to 28.98 mg kg^{-1} (fm) respectively. The higher concentrations of Cu in muscle tissue, liver and kidney of pigs i.e. (1.23, 5.94, 7.02 mg kg^{-1} respectively) allow us daily consumption of these without any limitation, since the maximum allowable daily limit intake of Cu, and Zn to the adult is 10 and 11 mg respectively (Codex Alimentarius Commission, 2001; Commission Regulation, 2006, 2008, 2011). In contract higher Zn concentrations in muscle tissue, liver and kidneys of the pig, i.e. (39.01, 18.44, 28.98 mg kg^{-1} respectively) shown that daily consumption of 282 g of muscle tissue or 597 g, or 380 g of liver complement the maximum daily Zn intake limit for an adult.

Accumulated amount of Cu in kidney (mean concentration 4.37 mg kg^{-1}) was not different statistically (Fisher's test $p > 0.05$) compared with the amount of Cu observed in liver (average concentration 4.19 mg kg^{-1}). However, Cu accumulation was higher in kidney and liver ($p < 0.05$) compared with the Cu accumulation found in muscle tissue of pigs (average concentration of 0.67 mg kg^{-1}) (Figure 1).

Furthermore, accumulation of Zn in muscular tissues of pigs (mean concentration of 23.71 mg kg^{-1}) was higher ($p < 0.05$) than the accumulation observed in kidney (average concentration of 20.04 mg kg^{-1}) and liver (average concentration of 17.25 mg kg^{-1}) (Figure 2). Accumulation of Zn was not statistically significant ($p > 0.05$) different between kidney and livers in pig (Figure 2).

It should be mentioned that both the liver and kidney of piglets appeared the same attitude towards the accumulation of Cu and Zn, while muscle tissues had a completely different attitude appearing stronger Zn accumulation.

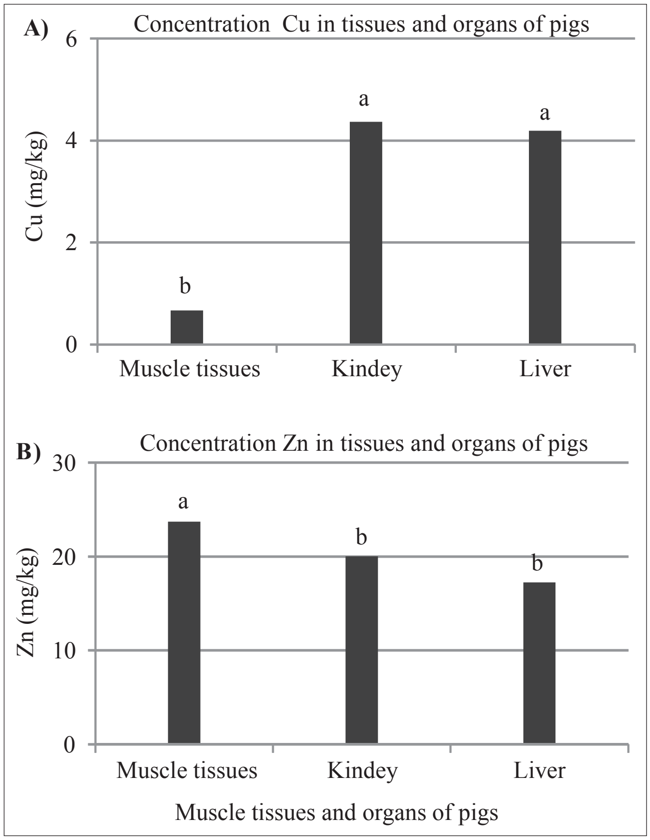

Fig. 2. Accumulation of heavy metals of Cu and Zn in muscle tissues (mg kg^{-1} fresh meat weigh after fat removal) and internal organs of pigs (mg kg^{-1} fresh meat weigh). Columns in each characteristic of each graph with the same letter do not differ significantly according to the Fisher's test (P=0.05). Data represents average means and standard error (SE) N=21. Cu=0.67±0.06, Cu=4.37±0.3, Cu=4.19±0.19, Zn=23.71±1.73, Zn=20.04±1.15, Zn= 17.25±0.21

Finally, the amount of Cu accumulation of muscle tissues and the examined internal organs of pigs had the following order: kidney = liver > muscle tissues and Zn, muscle tissues > kidney = liver.

Conclusions

Due to the high concentrations of heavy metals found in a large number of samples from the feed area of farms in Thessaly, our study suggests that feedstuff needs to be improved.

Furthermore this study showed contamination by heavy metals in a large number of samples of kidney and liver of pigs from the industrial area of Larissa, with a hazardous impact on human health. It is proposed to complement as little as possible kidney or liver, since 63 g of liver or 61.9 g of kidneys compilation complement the maximum allowable daily limit intake of Cr for an adult while, 282 g muscle tissue complement the maximum allowable daily limit intake of Zn for an adult.

Moreover, the detection of low accumulation of heavy metals in muscle tissues and internal organs of pigs except low heavy metal content in feedstuff, required low levels of heavy metals also in drinking water an in the environment.

References

Abou-Arab, A. A. K., 2001. Heavy metal contents in Egyptian meat and the role of detergent washing on their levels. *Food and Chemical Toxicology*, **39** (6): 593-599.

Adei, E. and K. Forson-Adaboh, 2008. Toxic (Pb, Cd, Hg) and essential (Fe, Cu, Zn, Mn) metal content of liver tissue of some domestic and bush animals in Ghana. *Food Additives and Contaminants,* **1** (2): 100-105.

Allen, S. E., H. M. Grimshaw, J. A. Parkinson and C. Quarmby, 1974. Chemical analysis of Ecological materials. *Blackwell Scientific Publications.*

Codex Alimentarius Commission (FAO/WHO), 2001. Food additives and contaminants. *Joint FAO/WHO Food Standards Programme*, ALINORM 01/12A, pp. 1-289.

Commission Regulation (EC), 2006. Setting Maximum Levels for Certain Contaminants in Foodstuffs. *Commission Regulation* No 1881/2006 of 19 December 2006, OJ L 364/5 of 20 December 2006.

Commission Regulation (EC), 2008. Amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. *Commission Regulation* No 629/2008 of 2 July 2008. OJ L 173/6 of 3 July 2008.

Commission Regulation (EC), 2011. Amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. *Commission Regulation* No 420/2011 of 29 April 2011 OJ L 111/3 of 30 April 2011.

Demirezen, O. and K. Uruc, 2006. Comparative study of trace elements in certain fish, meat and meat products. *Food Chemistry*, **32:** 215-222.

EC (European Commission), 2001. The Soil Protection Communication-DG Environment. Draft 2001. http://www.europa.eu.int /comm./environment /agriculture/pdf/ soilpaper2.pdf (accessed on 05/07/14)

EI-Salam, N. M. A., S. Ahmad, A. Basir, A. K. Rais, A. Bibi, R. Ullah and I. Hussain, 2013. Distribution of heavy metals in the liver, kidney, heart, pancreas and meat of cow, buffalo, goat, sheep and chicken from kohat market Pakistan. *Life Science Journal*, **10** (7).

European Food Safety Authority, 2009. Scientific opinion of the panel on additives and products on substances used in animal feed. *EFSA Journal*, **1043**: 1-69.

European Food Safety Authority, 2010. Scientific opinion on lead in food. *EFSA Journal,* **8**: 1570.

European Food Safety Authority, 2012. Cadmium dietary exposure in the European population. Scientific Report of EFSA. *EFSA Journal,* **10**: 2551.

Farmer, A. A. and A. M. Farmer, 2000. Concentrations of cadmium, lead and zinc in livestock feed and organs around a metal production centre in eastern Kazakhstan. *Science Total Environment*, **257** (1): 53-60.

Gorni, C., C. Garino, S. Iacuaniello, B. Castiglioni, A. Stella, G. L. Restelli, G. Pagnacco and P. Mariani, 2011. Transcriptome analysis to identify differential gene expression affecting meat quality in heavy Italian pigs. *Animal Genetics*, **42** (2): 161-171.

Ihnat, M. and L. Fernandes, 1996. Trace elemental characterization of composted poultry manure. *Bioresource Technology*, **57** (2): 143-156.

Li, Y. X. and T. B. Chen, 2005. Concentrations of additive arsenic in Beijing pig feeds and the residues in pig manure. *Resources Conservation and Recycling*, **45** (4): 356-367.

Maas, S., E. Lucot, F. Gimbert, N. Crini and P. M. Badot, 2011. Trace metals in raw cows' milk and assessment of transfer to Comté cheese. *Food Chemistry*, **129:** 7-12.

McBride, M. B. and G. Spiers, 2001. Trace element content of selected fertilizers and dairy manures as determined by ICP-MS. *Soil Science and Plant Analysis*, **32** (1-2): 139-156.

Moral, R., M. D. Perez-Murcia, A. Perez-Espinosa, J. Moreno-Caselles, C. Paredes and B. Rufete, 2008. Salinity, organic content, micronutrients and heavy metals in pig slurries from South-eastern Spain. *Waste Management*, **28:** 367-371.

Nicholson, F., S. Smith, B. Alloway, C. Carlton-Smith and B. Chambers, 2006. Quantifying heavy metal inputs to agricultural soils in England and Wales. *Water and Environmental Journal*, **20:** 87-95.

Ogundiran, M. B., D. T. Ogundele, P. G. Afolayan and O. Osibanjo, 2012. Heavy metals levels in forage grasses, leachate and lactating cows reared around lead slag dumpsites in Nigeria. *International Journal of Environmental Research*, **6** (3): 695-702.

Ryan, B. F., B. L. Joiner and J. D. Cryer, 2005. MINITAB Handbook: Updated for release 14, 5th edition.

Sager, M., 2007. Trace and nutrient elements in manure, dung and compost samples in Austria. *Soil Biology and Biochemistry*, **39** (6): 1383-1390.

Toor, G. S., B. E. Haggard and A. M. Donoghue, 2007. Water extractable trace elements in poultry litters and granulated products. *Journal of Applied Poultry Residues*, **16** (3): 351-360.

Varian, M., 1989. Flama Atomic Absorption Spectroscopy, Analytical Methods. *Varian Australia*, publ. №: 85-100009-00.

Wang, H., Y. Dong, Y. Yang, G. Toor and X. Zhang, 2013. Changes in heavy metal contents in animal feeds and manures in an intensive animal production region of China. *Journal of Environmental Science*, **25** (12): 2435-2442.

Wong, M. H., 1985. Heavy metal contamination of soils and crops from auto traffic, sewage sludge, pig manure and chemical fertilizer. *Agriculture Ecosystems and Environment*, **13:** 139-149.

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