



Effects of dietary lead exposure and graded levels of ascorbic acid supplementation on performance and haematology of broiler chickens

EB Ibitoye^{1*}, BR Olorede², BM Agaie³, A Ahmed¹, & AA Jimoh¹

¹Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine
Usmanu Danfodiyo University, Sokoto, Nigeria

²College of Agriculture, Ejigbo Campus, Osun State University, Nigeria

³Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine
Usmanu Danfodiyo University, Sokoto, Nigeria

*Correspondence: Tel.: +2347038228200, E-mail: emmavet2001@hotmail.com

Abstract

The effects of dietary lead acetate (LA) exposure on growth performance and haematological characteristics of broiler chickens and possible ameliorative effect of ascorbic acid (AA) were investigated. One hundred and twenty day-old broiler chicks were randomly divided into six treatment groups of 20 birds with two replicates. Six isonitrogenous and isocalories experimental diets were compounded and fed to birds for seven weeks: T₁ (control) received diet I (0mg of LA and AA/kg feed), T₂ received diet II (200mg LA/kg feed), T₃ received diet III (200mg LA and 50mg AA/kg feed), T₄ received diet IV (200mg LA and 100mg AA/kg feed), T₅ received diet V (200mg LA and 150mg AA/kg feed) and T₆ received diet VI (200mg LA and 200mg AA/kg feed). Finally, two birds per replicate were randomly selected, weighed and slaughtered. For haematology, blood samples were collected into labelled EDTA-bottles. PCV, RBC, WBC and Hb concentration were determined using Auto Haem analyzer. T₂ showed significantly (P<0.05) decreased body weight and dressed weight than the control. Although depressive effects of LA on body weight gain, feed intake and feed conversion ratio were significant. Birds in other treatments compared favourably with the control. Dietary lead significantly (P<0.05) reduced WBC in T₂, while it significantly (P<0.05) and non-significant (P>0.05) lowered haemoglobin and RBC in T₂ and T₆ respectively. The PCV was numerically lowered in T₂ and T₆. However, haematology of AA treated groups, especially those of T₃ and T₄ compares favourably with the control. In conclusion, dietary lead exposure negatively affects growth performance and haematology of broilers, which was ameliorated by as low as 50mg AA/kg diet supplementation.

Keywords: ascorbic acid, broiler, growth performance, haematology, lead acetate.

Introduction

Lead is a common cause of poisoning in domestic animals throughout the world. Cattle are the most susceptible livestock (Khan *et al.*, 2008). However, lead poisoning can occur in all domestic animals including horses, poultry and dogs (Khan *et al.*, 2008). Animals become intoxicated when they consume lead from contaminated feed and water. The main sources of contamination of feed by lead are soil, industrial pollution and agricultural technology as well as feed processing. A good source of lead contamination of poultry feed is bone and blood meals, majority of which comes from cattle. However, in cattle, highest lead accumulation has been reported to occur in bones (Heaney, 2000). Vegetables constitute essential components of the diet by contributing protein,

vitamins, iron, calcium and other nutrients, which are usually in short supply (Suruchi & Pankaj Khanna, 2011). However, vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on different parts of the vegetables exposed to the air from polluted environment (Zurera-Cosano *et al.*, 1989). It was reported that nearly half of the average ingested lead, cadmium and mercury in food is due to plant origin (fruit, vegetables and cereals). Other sources of lead contamination are leaded gasoline fumes (Genevieve & Greg, 1994); paints used in poultry equipment such as drinkers and feeders to prevent rusting, with either lead based paint or lead-free paint with leaded drying agent. Also, majority of litter material that comes from woods

[Click Here to upgrade to
Unlimited Pages and Expanded Features](#)

indiscriminate disposal of used batteries and its contents, grease and automobile oil filters in and around poultry houses in the study area.

Ingested lead has resulted in poisoning, poor performance and death in animals (McDowell, 1992; Gurer & Ercal, 2000). Stone & Soares Jr., (1976); Vodela *et al.* (1997), reported that, dietary lead poisoning in broiler chickens significantly reduced body weight and body weight gain. Erdogan *et al.* (2005) showed that 200 mg lead/kg diet reduced growth in term of body weight and body weight gain. Bakalli *et al.* (1995), also reported that feed conversion ratio was significantly poor at a level of 10 mg lead/kg feed. Lead accumulation in kidney and liver of broiler was reported by Erdogan *et al.* (2005), as well as by Khan *et al.* (1993), who stated that toxic doses of lead administered orally accumulate in the liver and this could be the source of lead poisoning to humans. In some cases, haematological parameters may provide an indication of lead intoxication. Among the major effects of lead poisoning is anemia, which results from inhibition of the heme synthesizing enzymes with concurrent elevation of protoporphyrin (Lee, 1981). This assertion was further buttressed by Osweiler (1996) who reported that lead slows down haemosynthesis through inhibition of enzymes. A hypochromic, regenerative anaemia was reported to occur in some affected birds poisoned with lead (McDonald, 1988).

According to studies (Stoys & Bagchi, 1995; Mateo *et al.*, 2003), lead has a potential to induce oxidative stress and acts as a catalyst in the oxidative reactions of biological macromolecules. Hence, the toxicities associated with lead might be due to oxidative tissue damage (Gurer & Ercal, 2000; Ercal *et al.*, 2001). To prevent peroxidative tissue damage, there are protective mechanisms *in vivo*, such as an enzymatic defense system (antioxidant enzymes) and free radical scavengers (antioxidants).

Ascorbic acid (AA) is a well-known antioxidant vitamin involved in several biochemical processes in biological systems. This vitamin breaks the chain of lipid peroxidation in cell membranes and scavenges free radicals such as reactive oxygen species (Carr & Frei, 1999; Kucuk *et al.*, 2003). The antioxidant function of these macronutrients could enhance immunity by preserving the functional and structural integrity of important immune cells, it lower concentration of lead in the blood and restored the levels of iron, calcium and zinc in the blood as well as the lipid balance. AA supplementation offered

protection to the cell from expansion or abnormalities in their structural features. Erdogan *et al.* (2005) reported that the addition of 100mg AA/kg diet tend to reduce the inhibitory effect of lead on growth in broilers. This study was conducted to evaluate the effect of various doses of AA, an antioxidant in ameliorating the inhibitory effects of dietary lead acetate on the haematology and growth performance of broiler chickens.

Materials and methods

Experimental Diets

Six isonitrogenous and isocalories experimental diets were formulated as shown in Tables 1 and 2. Tablets of vitamin C (Vitamin C, 100mg tabs, Michelle Laboratories Limited, Enugu, Nigeria) and lead in the form lead acetate salt (Lab Tech Chemicals, India) were crushed into powder. During feed composition, the smallest feed component by volume were weighed out and spread on a clean cement floor in the feed compounding vat. The next was salt and for those diets that contain lead acetate and or ascorbic acid, these were weighed using top loading balance and mixed with vitamin premix and minerals thoroughly, until a uniform ingredient was obtained. Other feed ingredients were individually weighed using a 20kg kitchen weighing scale and were spread on the clean floor in the feed compounding vat and were thoroughly mixed together manually. Each experimental diet was separately prepared in batches of 100% each and was packed into labelled bags and properly kept until required for use. The control diet I contained neither lead acetate nor ascorbic acid (0mg lead acetate/kg feed and 0mg ascorbic acid/kg feed), while diets II, III, IV, V and VI contained lead acetate at a fixed level of 200mg lead acetate/kg feed. Also diets II contained no ascorbic acid or 0mg ascorbic acid/kg feed. Other diets, i.e. diets III, IV, V and VI contained: 50; 100; 150 and 200mg ascorbic acid/kg feed respectively. These inclusions were done for both starter and finisher rations.

Experimental design

A Completely Randomised Design (CRD) was used. A total of 120 day-old broiler chicks were used. The birds were weighed and randomly distributed into six treatment groups of 20 birds per treatment; i.e. treatments I, II, III, IV, V and VI (i.e. T1, T2, T3, T4, T5 and T6). Each treatment group was replicated twice with 10 birds per replicate; the birds were raised using deep litter system and the experimental diets I, II, III, IV, V and VI were respectively fed to each treatment. Starter diets were fed from day-old to fourth week of age and finisher diets from fifth week of age until the end of the experiment. They were allowed ad libitum access to feed and water for a

[Click Here to upgrade to
Unlimited Pages and Expanded Features](#)

Performance Parameters: The body weight gain and feed consumption were determined weekly throughout the duration of the study. All the birds in a replicate pen were weighed collectively and divided by the total number of birds in that replicate pen, to determine the average weight per bird for the week. Similarly, the feed consumed by birds in each replicate of the treatment groups was also recorded on weekly basis, by subtracting the leftover at the end of the week from the quantity supplied for the week. The average feed consumed per bird was calculated by dividing the total feed consumed by the number of birds in that replicate pen. The average increase in weight gain per bird in a week was then calculated. This was carried out by subtracting the new week average weight per bird from the previous week. Feed conversion ratio was calculated by dividing the feed consumed per bird per week with average increase in weight gain per bird week. This was done for each treatment group as described by Oluyemi & Roberts (2000).

Haematological Parameters: At the end of the feeding trials, the birds were starved overnight to stabilize them. Two birds were randomly sampled from each replicate, weighed and slaughtered using Halaal/Kosher method of slaughtering. For haematology, blood samples were collected from severed jugular vein into labelled sterilized bottles containing Ethylene Diamine-Tetra-acetic Acid (EDTA). The packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and its differentials as well as haemoglobin (Hb) concentrations were determined using Auto Haem Analyzer (Via Guido

Borghetti 43, 21025 Comerio-Varese – Italy) as described by the manufacturer.

Statistical Analysis

Data obtained were subjected to one-way ANOVA and significant means were compared with post hoc test, using GraphPad InStat 3 software. Results were considered to be statistically significant when P values are less than 0.05 ($P < 0.05$).

Results

The effects of lead and ascorbic acid on body weight, body weight gain, feed intake and feed conversion ratio are presented in Table 3. Supplemental dietary lead significantly reduced body weight ($P < 0.05$), but its effects on body weight gain, feed intake and feed conversion ratio were not significant ($P > 0.05$) between treatment means. No clinical signs of lead toxicity were observed in the broilers administered lead. However, the body weight, body weight gain, feed intake and feed conversion ratio of ascorbic acid treated groups were statistically similar to that of control.

As shown in Table 4, the dietary treatment with lead significantly ($P < 0.05$) lowered WBC in T_2 ($66.88 \times 10^3/\text{mm}^3$) and haemoglobin which was significantly ($P < 0.05$) reduced both in T_2 (9.85g/dl) when compared with the control and other treatments. T_2 and T_6 produced numerical anaemia as their RBCs were below normal ranges. The PCV was numerically lowered in T_2 and T_6 . Numerically and statistically, all the haematological parameters of ascorbic acid treated groups, particularly those of T_3 and T_4 compares favourably with those obtained for the control group (T_1).

Table 1: Composition of Experimental Diet; Broiler Starter

Ingredients	Percentage composition (%)					
	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet VI
Maize	55.00	55.00	55.00	55.00	55.00	55.00
Groundnut Cake	30.00	30.00	30.00	30.00	30.00	30.00
Fish Meal	3.00	3.00	3.00	3.00	3.00	3.00
Blood Meal	3.00	3.00	3.00	3.00	3.00	3.00
Wheat Offal	5.00	5.00	5.00	5.00	5.00	5.00
Bone Meal	0.80	0.80	0.80	0.80	0.80	0.80
Limestone	2.50	2.50	2.50	2.50	2.50	2.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Premix	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Total (kg)	100	100	100	100	100	100
Lead Acetate (mg/kg)	-	200	200	200	200	200
Ascorbic Acid (mg/kg)	-	-	50	100	150	200
	Calculated values					
Crude Protein (%)	23.39	23.39	23.39	23.39	23.39	23.39
Metabolizable Energy (kcal/kg)	2881.21	2881.21	2881.21	2881.21	2881.21	2881.21

Click Here to upgrade to
Unlimited Pages and Expanded Features

Boiler Finisher

		Percentage composition (%)				
		Diet II	Diet III	Diet IV	Diet V	Diet VI
Maize	62.00	62.00	62.00	62.00	62.00	62.00
Groundnut Cake	30.00	30.00	30.00	30.00	30.00	30.00
Fish Meal	1.00	1.00	1.00	1.00	1.00	1.00
Blood Meal	1.00	1.00	1.00	1.00	1.00	1.00
Wheat Offal	3.00	3.00	3.00	3.00	3.00	3.00
Bone Meal	0.80	0.80	0.80	0.80	0.80	0.80
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Premix	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Total (Kg)	100	100	100	100	100	100
Lead Acetate (mg/kg)	-	200	200	200	200	200
Ascorbic Acid (mg/kg)	-	-	50	100	150	200
		Calculated values				
Crude Protein (%)	20.89	20.89	20.89	20.89	20.89	20.89
Metabolizable Energy (kcal/kg)	2981.79	2981.79	2981.79	2981.79	2981.79	2981.79

Table 3: The effects of lead (200mg/kg) and graded levels of ascorbic acid on performance of broilers

Parameters	Dietary treatments					
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
Initial body weight/bird (kg)	0.098	0.088	0.093	0.093	0.080	0.095
Final live weight/bird (kg)	2.05 ^a	1.58 ^b	1.95 ^a	1.76 ^a	1.81 ^a	1.73 ^a
Body weight gain/bird (kg)	1.952	1.492	1.857	1.667	1.730	1.635
Feed intake/bird/wk (kg)	0.488	0.444	0.489	0.471	0.468	0.463
Feed conversion ratio	2.046	2.367	2.230	2.389	2.264	2.135

ab: Means on the same row with different superscripts are significantly different ($P < 0.05$).

Table 4: Haematological Parameters of Experimental Birds.

Parameters	Dietary treatments					
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
WBC ($\times 10^3/\text{mm}^3$)	79.75 ^a	66.88 ^b	72.88 ^a	90.75 ^a	79.95 ^a	84.35 ^a
RBC ($\times 10^6/\text{mm}^3$)	2.38	1.93	2.21	2.67	2.10	1.95
PCV (%)	33.25	28.75	32.25	35.75	29.75	27.25
Haemoglobin (g/dl)	13.05 ^a	9.85 ^b	12.20 ^a	13.65 ^a	11.75 ^a	10.30 ^a

ab: Means on the same row with different superscripts are significantly different ($P < 0.05$).

Discussion

Dietary treatment with lead acetate in this study significantly ($P > 0.05$) decreased body weight in the experimental broilers, which is in agreement with previous findings by Morgan *et al.* (1975), and Erdogan *et al.* (2005). The reduction of body weight might be due to the interruption in absorption and metabolism of feed nutrients essential for health (Marchlewicz *et al.*, 2007). Also, ingestion of lead acetate at 200mg/kg feed did not result in significant ($P > 0.05$) decrease in the body weight gain of broiler chickens. This corroborate the result of Damron *et al.* (1969), who observed that broilers (4 weeks-old) were relatively resistant to lead poisoning at levels up to 2,000 mg Pb/kg body weight. However, this

result contradicts that of Morgan *et al.* (1975) and Erdogan *et al.* (2005) who reported that body weight gain was statistically lowered in the lead-treated group than that in the control and ascorbic acid-treated groups. This contradiction could be attributed to environmental factors such as temperature difference, differences in strain of birds used or the variation in dietary composition. On the contrary, birds fed lead plus ascorbic acid produced similar body weight and body weight gain like those of the control. Thus ascorbic acid addition to diet tend to reverse the growth depressive effect of lead in broiler chickens. This might be due to the ability of ascorbic acid to offer protection to the cell

[Click Here to upgrade to
Unlimited Pages and Expanded Features](#)

es in e as a gainst lead toxicity (Bhattacharjee *et al.*, 2003). This could therefore imply that, in contrast to the result of Erdogan *et al.* (2005), the adverse effects of dietary lead exposure on body weight gain though not significant, at 200mg lead/kg feed, could be ameliorated with even as low as 50mg ascorbic acid/kg supplementation in the diet. However, the ameliorative effects of ascorbic acid may not be dose dependent.

In agreement with the report by Erdogan *et al.* (2005), the effects of lead and ascorbic acid on feed intake and feed conversion ratio recorded in the present study were not significant. The findings of Bakalli *et al.* (1995), in terms of feed conversion ratio contradict the result of this study. This might be due to: different form of lead used, the present study used lead acetate while Bakalli *et al.* (1995), used lead sulphate and period of exposure, as Bakalli *et al.* (1995), exposed the birds for 42 days whereas this study exposed the birds for 49 days. However, birds treated with only lead without ascorbic acid revealed decrease feed intake, thus indicating toxic effect of lead and/or alteration in feed palatability resulting into lower average body weights in this group at the end of seventh week, whereas groups exposed to lead as well as varying levels of ascorbic acid revealed increased feed intake per kg gain with decrease in ascorbic acid supplementation. This indicated that an ameliorative effect of ascorbic acid on decreased feed intake due to lead, however, feed intake was best at 50mg ascorbic acid/kg supplementation.

References

- Bakalli RI, Pesti GM & Ragland WL (1995). The magnitude of lead toxicity in broiler chickens. *Veterinary Human Toxicology*, **37**: 15-19.
- Bhattacharjee CR, Dey S & Goswami P (2003). Protective role of ascorbic acid against lead toxicity in blood of albino mice as revealed by metal uptake, lipid profile and ultra structural features of erythrocytes. *Bulletin of Environmental Contamination and Toxicology*, **70** (6):1189-1196.
- Carr A & Frei B (1999). Does vitamin C act as a pro-oxidant under physiological conditions? *The Journal of the Federation of American Societies for Experimental Biology*, **13**: 1007-1024.
- Damron BL, Simpson CF & Harms RH (1969). The effect of feeding various levels of lead on the performance of broilers. *Poultry Science*, **48**: 1507-1509.
- Ercal N, Gurer-Orhan H & Aykin-Burns N (2001). Toxic metals and oxidative stress. Part I: Mechanisms involved in metals-induced oxidative damage. *Current Topics Medicinal Chemistry*, **1**: 529-539.
- Erdogan Z, Erdogan S, Aksu T & Baytok E (2005). The effect of dietary lead exposure and ascorbic acid on performance, lipid peroxidation status and biochemical parameters of broilers. *Turkey Journal of Veterinary and Animal Science*, **29**: 1053-1059.
- Genevieve D & Greg JH (1994). Toxins. In: *Avian Medicine: Principles and Application*. (WR Branson, JH Greg & RH Linda, editors) Wingers Publishing, Inc., Lake Worth, Florida. Pp 1030-1051.
- Gurer H & Ercal N (2000). Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radical Biology and Medicine*, **29**: 927-945.
- Heaney RP (2000). Lead in calcium supplements. *Journal of the American Medical Association*, **284**: 1432-1433.

According to the result of this study, the leukopenia observed in T2 could have been due to aplastic anaemia as a result of overwhelming blood poisoning from lead as well as infiltration of bone marrow by lead. Also, significant haemoglobin reduction of birds in T2 with consequent, non-significant lowered PCV and RBC clearly indicated retardation and/or inhibiting effect of lead acetate on haeme synthesizing enzymes (Lee, 1981; Osweiler, 1996). In accordance with the present findings, Khan *et al.* (2008) observed that following lead acetate administration, there was moderate decrease in haemoglobin and PCV. Similarly, Szymezak *et al.* (1983) observed that haemoglobin level was reduced after intoxication with lead acetate at the dose of 400mg/kg of the fodder. Also, Kamruzzaman (2006) observed that following lead acetate administration, there was significant decrease of RBC, WBC and haemoglobin content in rats. The WBC, RBC, PCV and haemoglobin of the ascorbic acid treated groups, especially those of 50mg ascorbic acid/kg feed were within the normal range and this illustrated the ameliorative effects of ascorbic acid on dietary lead exposure as it affects haematology.

In conclusion, the results obtained from the present study showed that, supplementation with ascorbic acid, even as low as 50mg/kg feed may be of immense prophylactic and therapeutic values in exposed broiler chickens. Ascorbic acid caused significant ameliorative effect on lead acetate-induced toxicity by improving the reduced growth performance and haematological values back to normal.

- pharmacology, BAU, Mymensingh.
- Khan MSH, Mostofa M, Jahan MS, Sayed MA & Hossain MA (2008). Effect of garlic and vitamin-B complex in lead acetate induced toxicities in mice. *Bangladesh Journal of Veterinary Medicine*, **6**(2): 203-210.
- Khan MZ, Szarek J & Markiewicz K (1993). Effects of oral administration of toxic levels of lead and selenium upon concentration of different elements in the liver of broiler chicks. *Zentralblatt für Veterinärmedizin. Reihe A*, **40**: 652-664.
- Kucuk O, Sahin N, Sahin K, Gursu MF, Gulcu F, Ozcelik M & Issi M (2003). Egg production, egg quality and lipid peroxidation status in laying hens maintained at a low ambient temperature (6°C) and fed a vitamin C and vitamin E- supplemented diet. *Veterinary Medicine Czech*, **1-2**: 33-40.
- Lee WR (1981). What happens in lead poisoning? *Journal of the Royal College of Physicians of London*, **15**: 48-54.
- Marchlewicz M, Wiszniewska B, Gonet B, Baranowska BI, Safranow K, Kolasa A, Glabowski W, Kurzawa R, Jakubowska K & Rac ME (2007). Increased lipid peroxidation and ascorbic acid utilization in testis and epididymis of mice chronically exposed to lead. *Biometals*. **20**:13-19.
- Mateo R, Beyer WN, Spann JW, Hoffman DJ & Ramis A. (2003). Relationship between oxidative stress, pathology and behavioural signs of lead poisoning in mallards. *Journal of Toxicology and Environment Health A*, **66**: 1371.
- McDonald SE (1988). Lead Poisoning in Psittacine bird. In: *Current Veterinary Therapy IX, Small Animal Practice*, (RW Kirk, editor). Philadelphia WB Saunders Co. Pp 713-718.
- McDowell LS (1992). Minerals in Animal and Human Nutrition. Academic Press Inc., California. Pp 361-364.
- Morgan GW, Edwins FW, Thaxton P & Parkhurst CR (1975). Toxicity of dietary lead in Japanese quail. *Poultry Science*, **54**: 1636-1642.
- North M & Bell D (1990). *Commercial Chicken Production Manual (4th edition)*. Van Nostrand Reinhold, New York, NY. Pp 456.
- Oluyemi JA & Roberts FA (2000). Poultry production in Warm Wet Climates. Mac-Millan press. Spectrum Books Limited, Ibadan. Pp 229.
- Oswailer GD (1996). Toxicology. Wilians & Wilkins, Philadelphia, Pp 191-197.
- Stohs SJ & Bagchi D (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radicals Biology Chemistry*, **18** (2): 321.
- Stone CL & Soares Jr JH (1976). The effect of dietary selenium level on lead toxicity in the Japanese quail. *Poultry Science*, **55**(1): 341-349.
- Szymezak J, Zechalko A & Biernat J (1983). Effect of fodder fat type on blood plasma lipids in mice intoxicated with lead. *Bromatologia Chemia Toksykological*, **16** (2): 98-94.
- Suruchi & Pankaj Khanna (2011). Assessment of heavy metal contamination in different vegetables grown in and around urban areas. *Research Journal of Environmental Toxicology*, **5**: 162-179.
- Vodela JK, Lenz SD, Renden JA, McElhenney WH & Kempainen BW (1997). Drinking water contaminants (arsenic, cadmium, lead, benzene and trichloroethylene). 1. Interaction of contaminants with nutritional status on general performance and immune function in broiler chickens. *Poultry Science*, **76**: 1474-1492.
- Zurera-Cosano G, Moreno-Rojas R, Salmeron-Egea J & Pozo Lora R (1989). Heavy metal uptake from greenhouse border soils for edible vegetables. *Journal of the Science of Food and Agriculture*, **49**: 307-314.