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HEAVY METALS CONCENTRATION IN SOIL, PLANT, EARTHWORM AND LEACHATE FROM POULTRY MANURE APPLIED TO AGRICULTURAL LAND

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Key words: contamination, organic residues, multi-species soil system

ABSTRACT

Heavy metals of livestock wastes (poultry manure) were studied. Heavy metals from two types of poultry manure (sawdust and straw bed) may represent a potential environmental risk for surface and groundwater. The test was made using a terrestrial microcosm, the Multi-Species Soil System (MS3) developed in the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, National Institute for Agricultural and Food Research and Technology). The results of heavy metals in soils showed higher statistically significant differences ($p \leq 0.001$) for Zn and Cd in straw and sawdust poultry manure amended soil. In the case of, Cd, Pb and Hg values were increased also for straw and sawdust poultry manure but did not show statistically significant differences. The presence of heavy metals, in the aerial parts of the wheat plant (*Triticum aestivum*), was studied and only Cu (sawdust poultry manure) and Hg (straw poultry manure) showed statistically significant differences ($p \leq 0.01$ and $p \leq 0.05$ respectively). The concentrations of Cd, Cu, Zn and Hg in earthworms showed significant differences ($p \leq 0.01$, $p \leq 0.05$ and $p \leq 0.001$ respectively). Cu concentration showed significant differences ($p \leq 0.01$) for straw poultry manure only. Finally, regarding the presence of metals in the leachates, only zinc, copper and nickel at 0 and 12 days showed statistically significant differences ($p \leq 0.0001$) between control and the different types of poultry manure. For copper and nickel also differences were observed at 12 days.

Palabras clave: contaminación, residuos orgánicos, sistema suelo de multiespecies

RESUMEN

Se estudiaron los metales pesados de residuos avícolas (gallinaza) partiendo de dos tipos de estiércol de aves de corral (cama de serrín y paja) ya que pueden representar un potencial riesgo ambiental para aguas superficiales y subterráneas. El ensayo se realizó utilizando un microcosmos terrestre, el sistema de suelo de múltiples especies (MS3), desarrollado en el Instituto Nacional de Investigación y Tecnología Agraria y

Alimentaria (INIA). Los resultados mostraron que los metales pesados que alcanzaron diferencias estadísticamente significativas ($p \leq 0.001$) fueron el Zn y el Cd en los suelos enmendados tanto con gallinaza con cama de paja como con cama de serrín. En el caso de, Cd, Pb y Hg hubo un incremento también en los suelos enmendados con residuo de ave (paja y serrín), pero no mostraron diferencias significativas. Con respecto a la presencia de metales pesados, en las partes aéreas de la planta de trigo (*Triticum aestivum*), se comprobó que sólo el Cu (gallinaza con cama de serrín) y el Hg (estiércol de paja de aves de corral) mostraron diferencias significativas ($p \leq 0.01$ y $p \leq 0.05$, respectivamente). Las concentraciones de Cd, Cu, Zn y Hg en las lombrices encontradas en suelos enmendados con gallinaza con cama de serrín mostraron diferencias significativas ($p \leq 0.01$, $p \leq 0.05$ y $p \leq 0.001$, respectivamente) y solo se encontró en el Cu diferencias significativas ($p \leq 0.01$) para las lombrices en gallinaza con cama de paja. Por último, solamente los metales pesados cobre y níquel recogidos en los lixiviados a los 0 y 12 días mostraron diferencias estadísticamente significativas ($p \leq 0.0001$) entre el control y los diferentes tipos de estiércol de aves de corral. Para cobre y níquel también se observaron diferencias a los 12 días.

INTRODUCTION

Excessive application of chemical fertilizer in agricultural soil had caused serious environmental problems, deterioration of soil physical structures, nutrients unbalance of soil, and water eutrophication. Livestock and poultry manure can be an alternative source of fertilizer in organic farming, where the use of anthropogenic chemicals is prohibited (Wong *et al.* 1999).

The utilization of poultry manure as an organic fertilizer is essential for improving soil productivity and crop production (Cooperband *et al.* 2002, Dikinya 2010). However, several problems raised from applications of manure, including the salt toxicity of manure to plants (Meek 1974) and accumulation of trace metals in plants may pose a health risk when humans or livestock consume them (Diaz-Barrientos *et al.* 2003).

So, further evaluation of application of manure, especially from intensive farming, should be given. However, it is not clear what the results are when these manures containing high concentrations of heavy metals are applied in agricultural soil, especially in a long term, because metal input through application of manures to soil will have a different behavior affecting soil chemistry and plant growth as well as metal uptake from the metals picked in soil as metal sulphate (Miyazawa *et al.* 2002, Walker *et al.* 2003).

While the use of organic wastes as manure has been in practice for centuries world-wide (Straub 1997) and in recent times (López Masquera *et al.* 2008), there still exists a need to assess the potential impacts of poultry manure on soil chemical properties and leachates and in particular evaluating the critical application levels (Delgado *et al.* 2010).

The multi-species- soil system (MS3), from two types of poultry manure (sawdust and straw bed), has also proved to be functional for assessing effects on earthworm, plants and microorganisms on an agricultural land (Delgado *et al.* 2012), and combined pollutants in contaminated sites (Fernández *et al.* 2005), MS3 can be used to monitor the mobility of metals in relation to biota (Alonso *et al.* 2006).

The aim of this study was to apply the multi-species- soil system (MS3) for study the heavy metals on soil, organisms (plants and invertebrates), and leachates after the application of two types of poultry manure (sawdust or straw bed) on an agricultural land.

MATERIALS AND METHODS

Multi-species-soil system

The MS3 is an artificial assemblage of soil macro-organisms lying on homogeneous columns of sieved natural soil (Fernández *et al.* 2004, Boleas *et al.* 2005) that allows the assessment of its effects. In this experiment PVC cylinders (20 cm inner diameter and 30 cm high) covered by a fine nylon mesh at the bottom, to avoid soil loss, were used. The columns were installed in a climate room with a light-dark cycle of 16-8 h (1200 lux ±13 % coefficient of variation CV), air conditioning (21±1 ºC) and 55-60 % humidity. The MS3 columns were saturated with spring water. After that, 30 plant seeds and 10 invertebrates were introduced. During the exposure period, the MS3 was irrigated to simulate 1000 mm rainfall/year (Carbonell *et al.* 2009).

Soil, poultry manure and organisms

The soil used in this study was collected from an abandoned soil at "La Canaleja", an experimental plant that belongs to INIA (35 km east of Madrid city) and was classified as a Typic Haploxeralf Calciorthid according to soil taxonomy criteria (Soil Survey Staff, 2003). A soil sample (0-30 cm) was air-dried, passed through a 2 mm sieve and analyzed following the standard soil test laboratory procedures of the Spanish Ministry of Agriculture, Fishing and Food (MAPA 1994).

The main physicochemical characteristics of the soil were: pH, 8.3±0.45; EC (dS/m), 0.21±0.02; Kjeldahl nitrogen (%), 5.8±0.13; organic matter (%) 17±4.3; Ca (mg/kg), 4058±2.5; Mg extractable (mg/kg), 168±6.4; Na (mg/kg), 50±15.8; P (mg/kg), 1.7±0.13 and extractable K (mg/kg), 15±4.6.

Poultry manure was supplied by Castilla-León farms located in the northeast of Spain. The physicochemical characterization of the poultry manure mixed with straw and sawdust shows a high organic matter content (%) (59.2±18.5 and 62.4±18.3 respectively), Kjeldahl nitrogen (%) (4.36±1.04 and 3.43±0.0 respectively). Neutral pH (7.32±0.35) for straw and alkali pH (8.27± 0.45) for sawdust poultry manure.

Table I shows the heavy metals of the poultry manure mixed with straw or sawdust poultry manure, whose parameters were determined by Standard Methods (APHA, AWWA, WPCF, 2005).

TABLE I. HEAVY METALS OF THE POULTRY MANURES USED IN THIS STUDY (mean ± standard deviation)

Heavy metals (mg/kg)	Sawdust poultry manure	Straw poultry manure
Zn	196 ± 6.21	287 ± 6.27
Cr	6.37 ± 2.08	1.50 ± 0.13
Cu	109.41 ± 12.81	31.79 ± 2.58
Ni	4.50 ± 0.29	3.21 ± 0.11
Cd	0.174 ± 0.023	0.108 ± 0.013
Pb	1.054 ± 0.054	0.42 ± 0.130
$Hg.10^{-3}$	4.42 ± 0.30	5.28 ± 2.92

The terrestrial organisms used in the MS3 were invertebrates and terrestrial plants. *Eisenia foetida* (maintained in culture for several generations in our laboratory) was chosen because it plays a key role in the maintenance of soil structure and in the regulation of soil organic matter dynamics (Lavelle *et al.* 1997). Certified seeds of the vascular plant Triticum aestivum, was kindly supplied by the Spanish Office of Plant Varieties. The species selected for this study are recommended by the OECD (OECD 2004).

MS3 experimental protocols

Poultry manure was applied in two rates: 9 t/ ha (poultry manure straw) and 10 t/ha (poultry manure sawdust) in an attempt to cover agronomic requirements. Three MS3 were used, the first one was filled with 8 kg of the control soil and the remaining two were filled with poultry manure-amended soil. The treatments and the control were performed in triplicate. The MS3 was saturated (spring water; 730-940 mL/MS3) and after 24 hours, 10 adult earthworms (*E. foetida*) with an average weight of 250-300 mg were placed on top of the soil. Finally, 10 wheat's seeds (*T. aestivum* L.) were introduced into each MS3. After the earthworms were placed on the soil column, the MS3 was irrigated with 100 mL of spring water. Every day, seeds germination was observed and MS3 was irrigated (spring water 100 mL) 5 days a week. At the end of the experimental period (21 days, cereal germination period) the MS3 systems were opened; top soil samples, aerial parts of developed plants, and earthworms were taken for heavy metals analysis. After 14 days, no leachates were produced yet and it was decided to keep the irrigation, in ten MS3, for two more days, until saturation (450 mL, spring water), in order to obtain an acceptable amount of leachates.

Metals analysis in soil and leachates

The concentrations of metals in soils were determined following EPA method 3051 (USEPA 1994a). A representative sample of 200 mg was digested in 8mL of HNO_3: water (1:1) concentrated nitric acid for 10 min using a laboratory microwave unit (ETHOS SEL Model, Milestone, Monroe, CT). High-purity MQ-water (Millipore Milli-Q-system) and analytical grade reagents were used. Standard metal solutions were prepared from concentrated stock solutions (Merck, Germany).

Leachates were acidified up to pH 2 and analyzed following EPA method 3015 (USEPA 1994b)

Metals analysis in plants and earthworms

Earthworms (*E. foetida*) were transferred to the laboratory, rinsed in tap water and maintained in plastic boxes with MQ-moistened filter paper for 24 h at constant darkness for depuration. The depurated earthworms were individually weighed to know the biomass increase/loss during the exposure period.

Plants were rinsed with MQ-water to remove external metals contamination, placed on filter paper, air dried and after that weighed.

Earthworms and plants were digested with 8 mL HNO_3/Milli-Q-water (1:1) using a laboratory microwave unit and analyzed using EPA method 3052

(USEPA 1996). Soils, plants, earthworm's solutions and the acidified leachates were analyzed for metals (Cd, Cr, Cu, Ni, Pb and Zn) by atomic absorption spectrometry graphite furnace AAS (GF-AAS) or flame atomic absorption spectroscopy (FAAS), depending on the concentration range, in a Perkin-Elmer Analyst 800 equipped with Zeeman-effect background correction. Mercury analysis was carried out in a direct mercury analyzer (DMA-80, Milestone Wesleyan University Middletown, CT, USA). Leachates were directly analyzed, although the determination of mercury in soil, plants and earthworms was done on the digested samples used for analysis of other metals.

Statistical analyses

Statistical differences for chemical properties of leachates, between poultry-manure amended soil treatments and the control, were assessed by analyzing the variance one-way ANOVA test and the least significant difference (LSD) multiple range test at confidence levels of 95 % and 99 % (SAS Institute 2001).

RESULTS AND DISCUSSION

Metals analysis in soils

The concentrations in soil for the seven selected metals in this experiment are summarized in **Table II**. The results were obtained at the end of the experimental period (day 21).

The values for Cd, Pb and Hg showed an increase in straw poultry manure amended soil (0.049 mg/kg, 8.0 mg/kg and 0.059 mg/kg respectively) with respect to the control values (0.039 mg/kg, 6.80 mg/kg and 0.05 mg/kg respectively) but were not statistical differences. The largest concentrations were observed for Cr and Zn and significant differences ($p \leq 0.001$) for straw and sawdust poultry manure were showed.

Regarding Cr, the results were similar for two types of poultry manure amended (7.20 and 7.30 mg/kg respectively) and finally, Zn was higher in straw than in sawdust poultry manure (25.0 and 21.0 mg/kg respectively). Increased concentrations of Cu and Zn in the surface horizons of soil receiving annual applications of PL (poultry litter) have been identified (Kingery *et al.* 1994). Copper and Zn concentrations in the surface of a soil profile that had received PL applications over 25 yr were higher than an unamended soil. Furthermore the results of this study suggest that Zn is fairly mobile in the profile. Using sequential extraction techniques, Cu was found to be mostly associated with the organic matter fraction in soils that had a 25 yr history of PL application (Han *et al.* 2000).

Nicholson *et al.* 2003 studied the contribution of different animal types to selected total metal (Zn, Cu, Pb and Cd) inputs to agricultural land in livestock. Heavy metals inputs to agricultural soils in England and Wales were: 1858 t/yr of Zn (47 % cattle, 27% pigs and 26 % poultry), 643 t/yr of Cu (33 % cattle, 55 % pigs and 12 % poultry), 48 t/yr of Pb (71 % cattle, 13 % pigs and 16 % poultry) and 4.2 t/yr of Cd (64 % cattle, 10% pigs and 26% poultry). Also Lei *et al.* (2009), analyzed heavy metals inputs to agricultural soils in China where livestock manures accounted for approximately 55 %, 69 % and 51 % of the total Cd, Cu and Zn inputs, respectively.

Study to evaluate the soil arsenic (As), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) enrichment that could result from the long term effect of poultry litter amendments and tillage practices on selected soil properties at the Alabama Agricultural Experiment Station, Belle Mina, AL, demonstrated that Cu and Zn did accumulate in the surface soil after 10 annual applications of poultry litter but not at phytotoxic

TABLE II. HEAVY METALS IN SOILS (mean ± standard deviation)

Soils	Treatments			
Heavy metals (mg/kg)	Control	Sawdust Poultry manure	Straw Poultry manure	*p*
Zn	$20.0^a \pm 0.9$	$21.0^b \pm 0.3$	$25.0^b \pm 0.1$	0.0004
Cr	$6.5^a \pm 0.6$	$7.2^b \pm 0.0$	$7.3^b \pm 0.0$	0.0006
Cu	$7.8^a \pm 1.4$	$6.0^a \pm 0.2$	$6.0^a \pm 0.1$	> 0.050
Ni	$5.2^a \pm 0.1$	$5.0^a \pm 0.3$	$5.5^a \pm 0.1$	> 0.050
Cd	$0.39^a \pm 0.08$	$0.40^a \pm 0.03$	$0.49^a \pm 0.07$	> 0.050
Pb	$6.8^a \pm 0.9$	$7.5^a \pm 0.5$	$8.0^a \pm 1.3$	> 0.050
Hg	$0.050^a \pm 0.006$	$0.051^a \pm 0.007$	$0.059^a \pm 0.018$	> 0.050

[a,b] Means with different superscripts are significantly different ($P < 0.050$) LSD test.
p = Probability values resulting from the analysis of variance

TABLE III. HEAVY METALS IN PLANTS (mean ± standard deviation)

Plants	Treatments			
Heavy metals (mg/kg)	Control	Sawdust Poultry manure	Straw Poultry manure	*p*
Zn	50.0[a] ± 3.1	47.0[a] ± 6.0	52.0[a] ± 1.6	> 0.050
Cr	-	-	-	-
Cu	10.4[a] ± 1.1	13.0[b] ± 1.2	11.0[a] ± 0.6	< 0.001
Ni	2.8[a] ± 1.2	1.7[a] ± 0.4	1.0[a] ± 0.3	> 0.050
Cd	0.10[a] ± 0.03	0.08[a] ± 0.03	0.08[a] ± 0.03	> 0.050
Pb	-	-	-	-
Hg	0.033[b] ± 0.005	0.030[b] ± 0.003	0.028[a] ± 0.000	< 0.050

[a,b] Means with different superscripts are significantly different ($P < 0.050$) LSD test.
p = Probability values resulting from the analysis of variance

levels in contrast to As, Pb, and Ni regardless of the tillage practices (Ngowari *et al.* 2013).

Metals analysis in plants

The metals concentrations in plants are shown in **Table III**. The results are on the same line as described by Alloway and Jackson (1991). They concluded that the metal uptake from soil to plant was very low and explained that sludge-born organic matter can introduce new binding sites to the soil and therefore present fewer risk for plants, as compared to soils without sludge addition. Uchimiya *et al.* (2012) and Wuana *et al.* (2012) also describe similar effects with soils amended with biocharts and biosolids of poultry manure. Cd, Cr, Ni, Pb and Zn concentrations in the aerial parts of the wheat plants (*T. aestivum* L) did not show statistically significant differences at any applied rate. Only the heavy metals Cu and Hg showed statistically significant differences. Copper concentration in plants (13.0 mg/kg) that grew on sawdust poultry manure amended showed significant differences ($p \leq 0.01$) and an increase versus control soil plants (10.4 mg/kg). This metal is important to the metabolism of the plants, given that the uptake of nutrients is regulated byit. It was expected that essential metal (Cu) presents a higher uptake, as it has been verified in other experiments (Miyazawa *et al.* 2002). The Hg concentration in plants that was lower on straw poultry manure (0.028 mg/kg) amended, showed significant differences ($p \leq 0.05$) and an increase versus control soil plants (0.033 mg/kg). This could be related with the findings of Alloway and Jackson (1991).

Metals analysis in earthworms

Table IV shows heavy metals concentrations in earthworms (*Eisenia foetida*). A statistically significant increase for cadmium ($p \leq 0.01$), zinc ($p \leq 0.05$) and mercury ($p \leq 0.001$) in the earthworms exposed to sawdust poultry manure was observed. This increment is extended to types, sawdust and straw, of poultry manure for copper ($p \leq 0.05$). On the contrary the chromium decreases in animals exposed to sawdust poultry manure (0.50 mg/kg) and to straw poultry manure (0.70 mg/kg), although no statistically signifi-

TABLE IV. HEAVY METALS IN EARTHWORMS (mean ± standard deviation)

Earthworms	Treatments			
Heavy metals (mg/kg)	Control	Sawdust Poultry manure	Straw Poultry manure	*p*
Zn	119[a] ± 3	140[b] ± 9	117[a] ± 5	< 0.050
Cr	1.0[a] ± 0.2	0.5[a] ± 0.3	0.7[a] ± 0.2	> 0.050
Cu	9.8[a] ± 1.2	15.0[b] ± 0.8	14.8[b] ± 0.9	< 0.050
Ni	-	-	-	-
Cd	0.8[a] ± 0.3	1.2[b] ± 0.4	1.0[a] ± 0.2	< 0.010
Pb	-	-	-	-
Hg	0.12[a] ± 0.01	0.15[b] ± 0.04	0.12[a] ± 0.02	< 0.001

[a,b] Means with different superscripts are significantly different ($P < 0.050$) LSD test.
p = Probability values resulting from the analysis of variance

TABLE V. HEAVY METALS IN LEACHATES (mean ± standard deviation)

Leachate		Treatments			
Heavy metals (µg/L)	Days 0	Control	Sawdust Poultry manure	Straw Poultry manure	*p*
Zn		68.18[a] ± 11.96	188.57[b] ± 19.69	192.70[b] ± 12.51	< 0.001
Cr		0.75[a] ± 0.22	0.74[a] ± 0.20	0.62[a] ± 0.01	> 0.001
Cu		27.86[a] ± 4.01	211.22[c] ± 15.25	115.35[b] ± 18.67	< 0.001
Ni		13.33[a] ± 10.10	111.90[c] ± 8.77	65.94[b] ± 1.75	< 0.001
Cd		0.043[a] ± 0.005	0.056[a] ± 0.028	0.033[a] ± 0.005	> 0.001
Pb		0.113[a] ± 0.037	0.119[a] ± 0.043	0.043[a] ± 0.040	> 0.001
Hg		0.010[a] ± 0.000	0.010[a] ± 0.000	0.010[a] ± 0.000	> 0.001
Heavy metals (µg/L)	Days 12	Control	Sawdust Poultry manure	Straw Poultry manure	*p*
Zn		33.98[a] ± 29.61	69.05[a] ± 18.24	49.75[a] ± 10.89	> 0.001
Cr		2.96[a] ± 0.13	5.12[a] ± 1.55	3.05[a] ± 0.21	> 0.001
Cu		34.82[a] ± 14.72	162.06[a] ± 3.98	80.29[a] ± 2.77	< 0.001
Ni		7.50[a] ± 0.01	114.70[a] ± 12.40	54.91[a] ± 3.41	< 0.001
Pb		0.383[a] ± 0.037	0.180[a] ± 0.095	0.343[a] ± 0.106	> 0.001
Cd		0.020[a] ± 0.010	0.013[a] ± 0.005	0.100[a] ± 0.020	> 0.001
Hg		0.010[a] ± 0.000	0.010[a] ± 0.000	0.010[a] ± 0.000	> 0.001

[a,b] Means with different superscripts are significantly different ($P < 0.001$) LSD test.
p = Probability values resulting from the analysis of variance

cant differences compared to the control (1.0 mg/kg) were found. Studies with in situ contaminated soils have confirmed that soil total metal concentration was a poor predictor of earthworm metal accumulation due to a number of modifying factors such as pH, organic matter and clay size particle content (Ma 1982). In order to avoid experimental constraints influencing the earthworm's response, Nahmani *et al.* (2007) suggest the necessity of performing field or terrestrial model ecosystem; bioaccumulation for earthworms showed high values for cadmium and mercury, and large differences when considering control earthworms versus earthworms cultured on poultry manure soil.

Metals analysis in leachates

Heavy metals concentrations in leachates are shown in **Table V**. Presence of copper, nickel and zinc, with 77.65 µg/L, 40.43 µg/L and 14.90 µg/L in leachates of straw poultry manure were observed, even after 19 days. Similar presence was observed regarding to zinc and nickel according to Gupta *et al.* (1997). This indicates that these metals (Cu, Ni, and Zn) may leach into groundwater. Trace elements Ni, Cu, and As were found to be readily soluble from PL (poultry litter). More than 70 % of Ni and Cu was in the cationic form, or bound in relatively labile complexes that dissociated to the cationic species. Hence, trace metal cations from PL are expected to be readily sorbed by soil mineral phases on land application of PL (Jackson *et al.* 2003).

In soils leached with PL leachate, Zn solubility from a contaminated soil was increased; however, in an uncontaminated soil, Zn from the PL leachate was retained by the soil matrix (Li and Shuman 1997).

A poultry litter application was conducted to examine field scale release and transport of trace elements from poultry litter into the subsurface. Field monitoring before and after litter application demonstrated increases in major ion, nutrient, and trace element concentrations in soil water after application, but concentrations of trace elements were all below regulatory standards. Using laboratory stepwise extractions of litter, calculated leaching rates of trace elements are fastest for As, followed by Cu and Zn (Oluyinka *et al.* 2012).

One way analysis of variance (ANOVA) for zinc, copper and nickel at 0 and 12 days showed statistically significant differences between the control and each type of poultry manure ($p < 0.001$). These differences also could be seen in copper and nickel at 12 days ($p < 0.001$).

CONCLUSION

The soil microscosm reproduced the specific conditions of agricultural arable land, such as MS3 system used in this experiment and offers a proper alternative for the study of heavy metals that are present in poultry manure residues.

The amended soil with poultry manure increased the concentration of zinc and cadmium in soils, copper in plants, and mercury in earthworms.

Finally, the presence of Zn, Cu and Ni in the leachates, in soils amended with poultry manure, was remarkable before 12 days.

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