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Heavy Metals in Oat and Soil Treated with Lime-Stabilized Biosolids and Reclaimed Wastewater

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Abstract

The potential risk of transfer of heavy metals from wastewater and biosolids to forage, livestock and ultimately to human is a serious issue. A field experiment was designed to assess the uptake of Cd, Cr, Ni and Pb from water and soil boat forage, and from oat forage to sheep tissues. Treatments consisted of application of lime-stabilized biosolids at the rates of 0, 25 and 50 Mg ha^{-1} dry weight basis along with a conventional rate of N fertilizer. Oat (*Avena sativa* L.) was planted in 300 m^{2}plots irrigated with reclaimed wastewater and twenty sheep were grazed. Kidney, liver and muscle were analyzed for metals concentrations. Soils treated with biosolids had significant less soluble Cr and Pb, but higher total Cr concentration than the control. Soluble and exchangeable forms in soil were higher for Cd (32.3%) than for Cr, Ni and Pb (5.4, 3.2 and 3.5%). Nickel and Pb concentrations were often higher than Cd, but Cr was not detected by the chemical analysis. Sheep ingested more Ni and Pb, but there were no significant treatment effects on metals concentrations in animal tissues. Cd concentration increased by 9.22% in kidney and 5.2% in liver for the sheep grazed in the treatment with 50 Mg biosolids ha^{-1}than the sheep not grazed on experimental plots. No metal bio-accumulation in sheep tissues was observed at toxic levels. Agricultural use of reclaimed wastewater and biosolids does not appear to represent a significant risk of metal contamination, but studies are needed to evaluate the risks associated with longer grazing periods, and plant care should be taken in expanding and implementing results of this study as biosolids characteristics vary from WTP to another.

Keywords: Reclaimed Wastewater; Heavy metals; Oat Forage; Sheep; Soil; Lime-Stabilized Biosolids

Introduction

The Paso Del Norte region of the U.S.-Mexico border is located with the Rio Grande river being the International boundary between New Mexico, Texas, and Chihuahua, Mexico. In this section of the Juarez Valley, Chihuahua, there are 22 rural communities directly exposed to pollutants in the reclaimed wastewater from urban sources during the last three decades [1]. In this region, almost 90% of agriculture and livestock production is based on reclaimed wastewater [2], and the main wastewater source come from Ciudad Juarez, Chihuahua, that has a population of 1.3 million. The two main wastewater treatment plants (WTP) utilize a secondary treatment system, and a total volume of 91 millionm^{3}was produced in 2012, also 105 255 m^{3}of anaerobically digested biosolids with 4% lime and 80% moisture content are generated per year [3].

All the treated wastewater has been used to irrigate 12250 ha agricultural area of irrigation district 04 Valle de Juarez in 2012. The crops grown were cotton (6091 ha), alfalfa (2477 ha), wheat (1557 ha), and others including pecan, rye grass, forage oat, sorghum, pistachio, and some vegetables (SAGARPA, 2013). The irrigation district was created originally to irrigate 20000 ha, and during some good productive years the agricultural area reached 17000 ha, because total volume of water available was 179 million m^3 a year from three sources: a) 74 million m^3 per year corresponds to 41.5% of irrigation water from Rio Grande river as result of the international agreement between Mexico and USA in 1906, b) water from private, and government wells that represent 22.9%, and c) reclaimed wastewater from Ciudad Juarez that means 35.7% of the total volume received in Juarez Valley [4]. Recently, the significant reduction in water availability from Rio Grande River is observed due to the drought conditions in states of Colorado and New Mexico, which is impacting irrigation in Juarez Valley, Chihuahua. Consequently, wastewater as a main source for irrigation is becoming important.

Wastewaters are dominant sources for irrigation water in Juarez Valley, which in turn affects the contaminant exposure of the urban population consuming local food products. Some local research has been conducted to quantify the impact on soils, forage crops, and animals. Some of the studies conducted in Juarez Valley irrigation District were focused on microbial pathogens in reclaimed wastewater. High levels were detected from 183 to >7000 *Giardia* cysts, and 9 to 762 *Cryptosporidium*oocysts per liter. Also, the presence of infectious *Cryptosporidium* was found, and wastewater contained *C. parvum* bovine (zoonotic) genotype and human-specific *C. hominis* sub genotypes. In this research, there was no evidence of human-to-animal transmission of *Cryptosporidium* or *Giardia*zoonoses [5].

Most studies regarding biosolids and wastewater at the Juarez Valley have focused on monitoring in-field contaminants affecting plant and animal yield functions, but have not addressed contaminant transfer. Heavy metals, plant nutrients, and microbial pathogens have been measured in wastewater in other studies as well [6,2,7]. Also, biosolids generated in Ciudad Juarez, Chihuahua, México were

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evaluated on agricultural soils to improve Oat, Sorghum and Rye grass production [8]. This body of work suggests that heavy metals and microbial pathogens in wastewaters, soil and biosolids could represent a high risk, but none of these studies verifies metal transfer from soil and water to plants, animals and ultimately human as we move down the food chain.

Environmental Protection Agency [9], and the Mexican legislation include Arsenic (As), Cadmium (Cd), Copper (Cu), Chromium (Cr), Mercury (Hg), Nickel (Ni), Lead (Pb), and Zinc (Zn) as heavy metals that need to be monitored in biosolids due to their potential impact on health. Nickel and Pb concentrations in biosolids generated in Juarez are almost twofold greater in comparison to those concentrations in biosolids from El Paso, TX. Cd and Cr concentrations are also high in the biosolids produced at Juarez [8,10]. Generally, characteristics of biosolids depend on the sources of wastewater and WTP they are produced [11]. As the biosolids characteristics vary from WTP to another plant care should be taken in expanding and implementing results of this study in biosolids produced in other WTP [12].

Other trace elements detected in wastewater and biosolids at Juarez are B, Cu, Fe, Mn, Mo, and Zn [13]. However, they are essential mineral nutrients for crop production. In contrast, Cd and Pb have no known physiological activity [14].

Field soils in Juarez are high in Pb concentrations [15,16]. High Pb, Cr, and Ni concentrations were also found in untreated effluent used for irrigation [10,15,16]. Particularly, Cd, Cr, Ni and Pb have been considered the metals that represent a current risk to the environment at Juarez Valley, Chihuahua, Mexico. Studies have shown that these metals are generally bound on soil constituents, do not easily leach from the soil, and are not readily available to plants [17]. Soil pH is factor controls heavy metal uptake by plants. Only in moderately to strongly acid soils is there significant vertical metal movement in soils. Alloway [18], demonstrated that Cd concentrations in oats decreased with increasing soil pH value.

Sheep ingestion of heavy metals from soil can vary from 0.5% to 24% of the total diet depending on the grazing conditions. Decreasing forage supplies increase the ingestion from soil [19]. Soil ingestion is recognized as potentially the most important route for the entry of heavy metals into body tissues of grazing livestock [20]. Critical concentrations of heavy metals in plants are 5-10, 1-2, 20-30, and 10-20 $mgkg^{-1}$ for Cd, Cr, Ni and Pb, respectively, and for animals are 0.5-1, 50-3000, 50-60, and 10-30 $mgkg^{-1}$ in dry matter for Cd, Cr, Ni and Pb, respectively [21].

There are general guidelines for assessing Cd and Pb exposure and toxicity of cattle and sheep [22]. Normal concentrations are 0.02-0.05 mg $Cdkg^{-1}$ fresh weight (FW) in liver, 0.03-1.0 mg $Cdkg^{-1}$FW in kidney, and 0.1-0.5 mg $Pbkg^{-1}$ FW in liver and kidney. High concentrations are considered 1.5 to 5 mg $Cdkg^{-1}$ FW, and 2 to 4 mg $Pbkg^{-1}$ FW. However, they are not high enough to harm animal health. Cd produces a cumulative intoxication which means that Cd concentration in adult animals are greater than in young animals [23]. The highest Cd concentrations in sheep tissues are stored in intestine, kidney and liver [24-26,22,20]. Maximum Cd concentration allowed for sheep diet is 0.5 $mgkg^{-1}$ [27], and the ceiling limit in kidney is 1 $mgkg^{-1}$ fresh weight [24,28,23].

Sheep fed with 100 mg $Pbkg^{-1}$ for 100 days did not have effect on the daily weight in food consumption, but there was an increase of Pb in liver and kidney. Calcium reduces Pb absorption; however, if there is more than 10 $mgkg^{-1}$ in liver, it can reach intoxication level [29]. Particularly, young sheep trend to cumulate more Pb in liver than old animals, the concentration of Pb reported is 0.09 $mgkg^{-1}$ [30], The maximum levels authorized by the Economic Europe Community is 2 mg $Pbkg^{-1}$ [22], and the maximum concentration permitted in diet (food) for sheep is 30 $mgkg^{-1}$ [27].

Chromium toxicity is not common because it is absorbed at very low amounts in the digestive system, its concentration in tissues are lower than 0.1 $mgkg^{-1}$ [22]. Nickel toxicity depends on the chemical form of the metal, type of animal, reproductive stage, and concentration in the diet. The concentration reported in animal tissues is lower than 1 $mgkg^{-1}$, but 50 $mgNikg^{-1}$ in animal food is considered toxic for sheep and bovines species [29,27]. Spears et al. [31], indicated that small increases in dietary Ni (5 $mgkg^{-1}$ dry matter) can cause six fold increase in Ni levels in the bovine kidney to 0.3 $mgNikg^{-1}$ DM, suggesting a gross dietary excess. O'Dell et al. [32], reported that kidney concentrations reach 38 $mgNikg^{-1}$ dry matter in toxicity, but the predominant route of excretion is via the feces.

The potential magnitude of heavy metal exposure in forage and livestock production using wastewater is not fully known. Therefore, the objectives of this study were to determine the potential risk of heavy metal transfer from wastewater, biosolids, and soil to oat plants and sheep tissues.

Materials and Methods

A field experiment was conducted at the Ejido San Isidro, Juarez Valley, Chihuahua, Mexico (Figure 1). The experimental site reflected actual growing conditions in managed agricultural areas at the Border of Mexico and USA. The soil at the site is a Typic Torrifluvent with clay texture, pH of 8.38 (1:1 soil/water ratio), EC of 2.2 dSm^{-1}, Olsen P of 264 $mgkg^{-1}$, total N of 19.6 gkg^{-1}, N-NO_3^- of 28 $mgkg^{-1}$, and organic matter of 1.67% in the top 15 cm of soil. The baseline data on irrigation water, biosolids, and soil was collected to characterize Cd, Cr, Ni and Pb concentrations. The site previously never received biosolids, but it

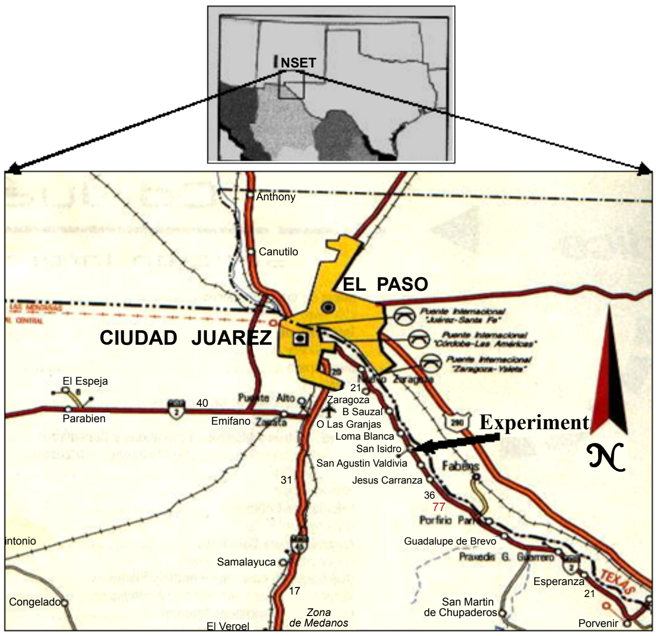

Figure 1: Location of the field experiment at the Juarez Valley, Chihuahua, Mexico.

was irrigated for several years with untreated municipal wastewater. The water quality parameters of untreated municipal water showed that it had pH of 7.35 ± 0.38, electrical conductivity of 1569 ± 120 µS m^{-1}, sodium adsorption ratio of 4.66 ± 304, total hardness 271.5 ± 19.5 mg L^{-1}, alkalinity 365 ± 53 mg L^{-1}, $NO_3$0.092 ± 0.07 mgL^{-1}, P8.92 ± 2.8 mgL^{-1}, Al 3.62 ± 5.8 μgL^{-1}, Fe 2.19 ± 2.9, Mn0.1165 ± 0.040 μgL^{-1}, Zn 0.2443 ± 0.1 μgL^{-1}, As 0.0075 ± 0.002 μgL^{-1}, and Pb0.0090 μgL^{-1} [13]. Soil was amended with lime-stabilized biosolids at rates of 0 (control), 25, and 50 $Mgha^{-1}$ on a dry weight basis. A fourth treatment with commercial fertilizer (ammonium sulfate) was included at a conventional rate for forage oat (120 kg N ha^{-1}). The fertilizer contained 0.66 and 0.21 $mgkg^{-1}$ Cr and Pb, respectively. The low biosolids rate was based on the agronomic rate reported by Flores et al. [8], for a representative soil cultivated with sorghum forage at the Juarez Valley. The high rate was meant to introduce more nutrients, pathogens and heavy metals to the soil-plant-animal system.

The experimental design was a one-way layout with plots arranged in a completely randomized block design with four replications. Plot size was 300 m^2 (15m x 20m), and was based on the area needed to feed five sheep in between 28-day irrigation cycles. Forage Oat *(Avena sativa L.*) was planted at the test site and managed according to the recommended agronomic practices following official guidelines for the Juarez Valley [33].

To evaluate potential contamination transfer in an agricultural setting, wastewater used for irrigation, soil, and oat forage were collected immediately after irrigations for a total of four sampling periods: prior to putting the sheep onto the plots (on Sep 8, 2003), after the first irrigation (on Oct 17, 2003), mid-point irrigation (on Dec 3, 2003) and last irrigation (on Apr 19, 2004). A second experimental evaluation was conducted from March to May 2004. Both periods of evaluation were considered as maximum risk dates for microbial transfer and heavy metal accumulation because wastewater was used for irrigation.

More intensive soil and plant tissue sampling was scheduled during the middle and at the end of the growing season to evaluate surface leaf debris for metal content. Changes in field moisture conditions (gravimetric determination), forage growth (crop height and yield), and observations on animal grazing habit were recorded. Replicated soil samples were collected at the surface from 0 to 5 cm and 5 to 15 cm depth. The above ground portion of forage was collected near the soil sampling sites three times during the evaluation period because sheep have contact with soil surface during feeding, and metals from biosolids remains on this soil depth.

Initially, three sheep were selected that were not grazed on the experimental plots to determine heavy metal profiles in muscle, liver, and kidney tissues. Five sheep were assigned to each soil treatment (5 sheep x 4 treatments = 20 sheep), beginning in the first experimental block. This number of Pelibuey sheep is usually used in experiments for feeding, and it varies from 4 to 12 in current studies [34]. Feeding pens (300 m^2) were constructed to corral grazing sheep. Sheep were rotated every seven days among experimental blocks for each irrigation cycle in two growing periods. This insured sheep have sufficient forage for grazing. After rotation, graze plots were mowed to ensure uniformity. Sheep were marked with ear tags and paint markers on the body to identify each of them by treatment.

Animals had a period out of the field before the start of the second evaluation period, and sheep were fed with supplement of alfalfa during this period. Sheep were assigned to each plot treatment at 7 a.m. for grazing, at 6 p.m. sheep returned to the small corral to spend the night and at that time they were provided with small amount of salt (minerals) and clean water.

Animals were weighed every15 days and were slaughtered at the end of the field experiment to collect samples from kidney, meat, and muscle. Samples were stored in a freezer and sent to the Laboratory at the Universidad Nacional Autonoma de Mexico (UNAM) in Mexico City. Sheep tissue samples were prepared by digestion with nitric acid and perchloric acid, and heavy metals were determined by atomic absorption spectroscopy.

Soil and forage were collected with shovels and clippers, respectively that have been chemically-sterilized with acetone, double-rinsed with deionized water, and dried to reduce extraneous contamination. Forage and soil samples were packaged in plastic bags and placed on ice for transport to the laboratory at Texas A&M University at El Paso, TX. A total solid of leaf surface debris was quantified on freshly collected forage. Debris was removed from tissue by extraction, and dried for weighing. A portion of the forage was washed in 0.1 M HCl followed by two rinses in deionized water to remove surface debris. Another portion remained unwashed to simulate the actual ingestion of grazing sheep. Both washed and unwashed plant tissue were dried in a forced-air dryer at 65°C for 72 hours, ground and passed through a 40-mesh screen. Samples were acid-digested with nitric and perchloric acids using EPA 200.2 protocols for heavy metal determinations using inductively-coupled plasma (ICP) spectroscopy [35]. Soil samples were air-dried and sieved prior to preparation for analytical determinations (Methods of Soil Analysis, Part 3). Total and sequential extractions for heavy metals were determined with ICP spectroscopy following U.S. EPA protocol 2007 [35].

Statistical Analysis

The data for concentrations of heavy metals in soil samples at two depths for total and fractionated forms, total metal concentrations in washed and un-washed oat tissues, total metals in sheep tissues (liver, kidney and muscle) were analyzed using analyses of variance (ANOVA) with the statistical package SAS [36]. Pearson correlation coefficients were used to determine significant associations between all variables were analyzed; and treatments means were compared by LSD test.

Results and Discussion

Reclaimed wastewater

Total heavy metal concentrations in irrigation water showed a large variation during the experimental period (Figure 2). The concentrations varied in the orderPb>Ni>Cr>Cd from May to November 2003, and Ni>Cr>Pb>Cd from January to April 2004.Cdconcentration was always<0.57 μgL^{-1}, Cr concentration varied from 0.41 to 165.33 μgL^{-1}, Ni from 5.3 to 259.34 μgL^{-1}, and Pb from 3.42 to 44.32 μgL^{-1}. For the same irrigation canal, Palomo et al. [13], reported concentrations of 0.5, 10, 20 and 9 $\mu gL^{-1}$for Cd, Cr, Ni and Pb, respectively. However, this study shows that all concentrations were lower than the ceiling limits of 200, 1000, 2000 and 500 μgL^{-1} for Cd, Cr, Ni and Pb, respectively according to the Mexican regulations [37] for wastewater.

The total amount of heavy metals added from wastewater to soil after eight irrigations were 399, 226, 223 and 0.4 gha^{-1} of Ni, Cr, Pb and Cd, respectively. Most of the water used for irrigation during the period from October to March is black partially treated water and that could be the cause of the variation in concentrations of metals in wastewater. The high concentrations may be caused by uncontrolled discharges

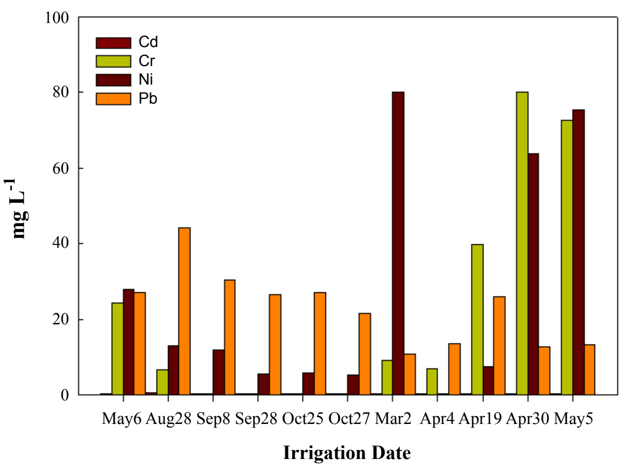

Figure 2: Maximum values of trace metals measured in irrigation water along the experimental period at the Juarez Valley in years 2003 and 2004.

or the municipal program requiring industries to clean pollutants from their wastewater before releasing it to municipal wastewater treatment plants may not be working. Other chemical characteristics of wastewater include pH of 7.21± 0.25, electrical conductivity of 1.614 ± 0.25 dSm^{-1}, N-$NH_4^+$of 26± 7 mgL^{-1} and $N0_3^-$-N- of 0.17± 0.3 mgL^{-1}. Inorganic N indicated that 27 kg ha^{-1}was added to soil at each irrigation, satisfying the N demand of oat plants.

Biosolids

Total heavy metal concentrations in biosolids were 1.29, 32.94, 5.34, and 21.04 mgkg^{-1} for Cd, Cr, Ni and Pb, respectively. A similar study showed concentrations of 1.09, 8.54, 11.08, and 12.5 mg kg^{-1} for the same metals in lime stabilized biosolids, and concentrations of 2.45, 50.2, 25.7, and 134 mg kg^{-1} in anaerobically digested biosolids, respectively [10]. Chemical fractionation analysis showed that Cd and Ni had 80 and 89% in insoluble or residual form, but Pb had 14.2% in soluble and exchangeable forms. Other studies at the same agricultural area have reported concentrations of 10.63, 40.33, 26.52 and 22.15 mg kg^{-1} Cd, Cr Ni and Pb for biosolids collected at the same wastewater treatment plant [38]. According to the Mexican Legislation [37] and U.S.A. regulations in U.S. EPA part 503 [9], the ceiling limits for Cd, Cr, Ni and Pb are 39, 1200, 420 and 300 mgkg^{-1}of dry biosolids. Therefore the concentrations found in this study were much lower than the regulatory values. This suggested that biosolids can be used in agricultural soils because with regarding to heavy metals content, they do not represent a risk.

Biosolids used in this study were a product of advanced primary water treatment and stabilized with 20% lime. The quality depends on the type of compounds present in wastewater and treatment technology. The sewage treatment method used in this WTP consisted of removing organic and inorganic solids by the physical mechanism of sedimentation and flotation. Approximately, 25 to 50% of the incoming bio-chemical oxygen demand, 50 to 70% of the total suspended solids, 65% of the oil-grease, and some organic nitrogen, organic phosphorus and heavy metals associated with solids are removed during this wastewater treatment [39].

The biosolids rate used in this study was 50 tha^{-1} biosolids dry weight, and the amount of metals added to the soil with this rate were 64.5, 1647, 267 y 1052 gha^{-1} of Cd, Cr, Ni and Pb, respectively. The concentration in biosolids varied from Cr>Pb>N>Cd. Chemical characteristics of biosolids were determined and showed a pH of 9.14, EC of 3.53 dSm^{-1}, and TKN of 1.82%, organic carbon of 9.2%, P of 1,393 mg $P0_4$ -Pkg^{-1}, Ca of 4,094 mgkg^{-1}, Mg of 12.8 mgkg^{-1}, and Na of 233 mgkg^{-1}. Other studies using the same type of biosolids showed similar values for pH from 9.1 to 12, EC from 3.4 to 8.2 dSm^{-1}, TKN from 1.88 to 3.0 %, Ca of 1,480 mgkg^{-1}, and Na of 69.9 mgkg^{-1} [6,40].

Soil

Total heavy metal concentrations in soil before application of biosolids were: 1.93, 13.9, 13.41, and 11.9 mgkg^{-1}for Cd, Cr, Ni, and Pb, respectively. In an earlier study, Flores et al. [8], reported 0.43, 14.27, 15.47, and 14.7 mgkg^{-1}of Cd, Cr, Ni, and Pb for a clay soil near the present experimental site. Assadian et al. [15], also found average values of 0.4, 10, 7, and 6.5 mgkg^{-1}of Cd, Cr, Ni, and Pb in a soil cultivated with alfalfa at San Isidro, Juárez. The concentrations given above are within the range for agricultural soils and varied in the orderCr>Ni>Pb>Cd (Figure 3).This order of variation is similar to the order of concentrations observed in wastewater (Figure 2).

There were no significant differences among treatments for the total heavy metal concentrations in soil three months after biosolids application ($P>0.05$). But, significant effects of treatments were detected on soluble soil Cr and Pb ($P<0.01$). Biosolids treatments had less than half Cr and Pb soluble fraction concentrations (0.388 mgkg^{-1}) compared to the control and fertilizer treatments (0.828 mgkg^{-1}). At the end of the study, as small treatment effect on total Cr concentration in soil was also observed. The control had 11.21 mg Crkg^{-1}while biosolids treatments contained 11.98 mg Crkg^{-1}. This increase can be explained by the less soluble fraction in the amended soil. In most of the cases, there was not significant effect of soil depth on metals concentration; however, 5-15 cm soil depth had lower concentrations than 0-5 cm depth, probably as a result of metals added with wastewater, sheep feces, or atmospheric deposition.

Forage yield and tissue metal concentrations

No significant differences in mean yield and plant height were observed among treatments in the analysis of variance at the first harvest for both 2003 and 2004 years (Figure 4). But, the average LSD test showed that fertilizer treatment had a significant effect on

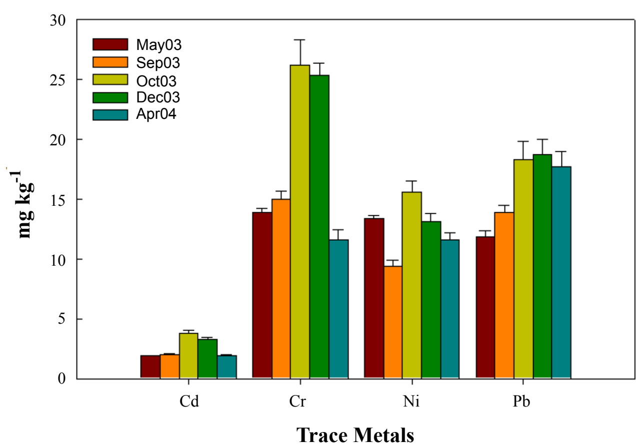

Figure 3: Average concentration of trace metals in soil 0 to 15 cm for five collection dates at the field experiment.

dry and wet forage yield in a second harvest in 2003. Similarly, LSD mean comparisons showed that biosolids treatment with 25 Mgha^{-1} significantly increased dry matter yield (950 kgha^{-1}) and water content in forage (82%) at the second harvest in 2004. Plant height varied from 24.8 to 36.2 cm when forage was harvested to measure yield and sheep were grazed during the entire study.

The small differences in dry matter yield of oat forage can be explained by the large amount of N supplied by irrigation water (27 kgha^{-1} per irrigation) and the high concentration of available phosphorus in soil (300 mgkg^{-1}). The lack of biosolids and fertilizer treatments effects on dry matter yield have also been reported by Flores et al. [8] and Flores et al. [41] for the Juarez Valley.

Heavy metal concentrations in plant tissues were not significantly different among treatments (*P*>0.05) for both washed and unwashed samples during 2003 and 2004 sampling periods. Coefficients of variation were high and may explain the lack of significance, mainly for the unwashed samples that contained soil or debris at the foliage. The variation in the washed material can be attributed to the plant tissue as a result of different uptake by oat plants. Concentration of metals for washed samples at the middle of the second period in 2004 was 0.07, 0, 6.69, and 5.88 mgkg^{-1} Cd, Cr, Ni, and Pb (Figure 5). Unwashed samples had 0.09, 0.53, 6.84, and 6.31 mgkg^{-1} for the same metals.

Differences can be attributed to the soil or particles in contact with the oat leaf and stem. The supplement (alfalfa) used to feed the sheep when they were out of the experimental plots had 0.21, 0.6, 1.73 and 5.15 mgkg^{-1} of Cd, Cr, Ni and Pb, respectively. Lead concentration was 10.99 mgkg^{-1} in the most common weed, Knotweed, found at the experimental site also named commonly erect Knotweed or mouse ear, which was almost double compared to oat samples (Figure 5). Although, the weed population was high at the experimental plots, sheep were selective for oat plants, and weed consumption was very low, but animals appeared to eat low amounts of soil when density of oat plants reduced as a result of animal feeding in some periods of the trial.

Oat is specie with low capacity to uptake Cd and Pb compared to other plants known a shyper-accumulators or indicators for heavy metals. For instance, 0.21 mg Cdkg^{-1} and 2.28 mgPbkg $^{-1}$ are reported in oat grain and 6 mg Pb kg $^{-1}$ in leaf [42]. The Pb concentrations are similar

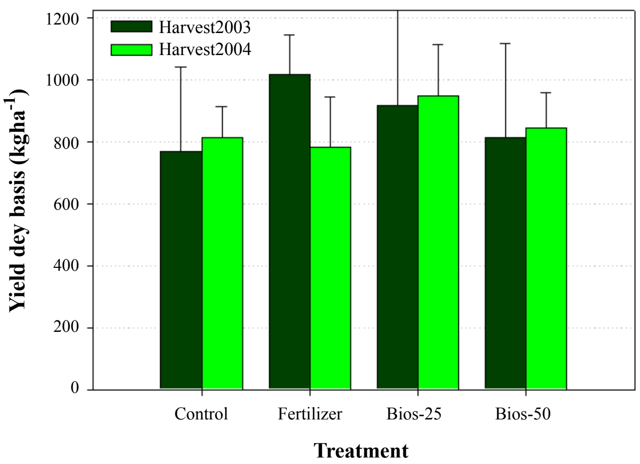

Figure 4: Oat forage yield in dry basis measured before graze the sheep in the treatments evaluated at the Juarez Valley in years 2003 and 2004.

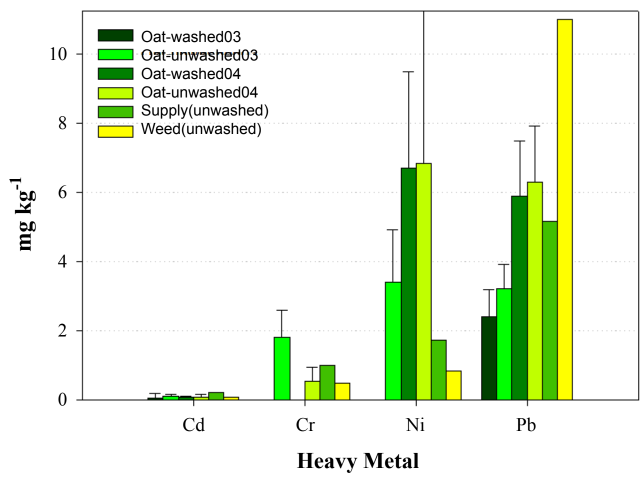

Figure 5: Trace metals content in oat forage, supplement and weed for washed and unwashed samples harvested.

to the result found in this study (Figure 5). The small concentrations of metals found in oat tissues can be explained by the type of plant, but also by the low solubility of metals in clay soils and the alkaline pH observed at the soil used in this study.

This result suggests that increases in soil Cd, Cr, Ni or Pb caused by water and biosolids were significantly related with increases in Cd, Cr, Ni or Pb. Correlations between heavy metal concentrations in washed plants (oat forage) and soil at 0 to 15 cm depth were not significant (P>0.05), only Ni_{plant} was associated with Cr_{soil} and Pb_{soil} (r=0.66 to 0.79). A significant correlation was observed for P_{plant} and Cd_{plant} (r=0.68). However, increases in P_{plant} were associated with decreases of Cd_{soil} and Cr_{soil} (r=-0.69).

The total feed intake by sheep included oat forage, small amounts of mineral supplement, and dried alfalfa. Although soil and weed were also important sources of heavy metals, they were not quantified directly but were partially included in the calculations of unwashed plant samples. Metal intakes by sheep were based on 1.5 kg forage dry basis or 8.3 kg wet basis with 82% water content per day per sheep. Previously, we indicated the lack of significant difference for metals content in plant tissues which means that metal intake by sheep was not statistically different among treatments.

The higher amounts of Pb than Ni metals were ingested; but Cd ingestion was less (Table 1). On average, sheep ingested 0.23, 1.37, 4.29 and 7.77 mgday^{-1} Cd, Cr, Ni, and Pb, respectively, from oat forage, alfalfa and salts given to sheep as a supplement. The concentration varied in the orderPb>Ni>Cr>Cd which is similar to the series showed for irrigation water in this study. Regarding the soil present in foliage (debris), sheep consumed 14, 95, 64, and 95 mg Cd, Cr, Ni, and Pb per year. Our estimates were based on 4.39 g soil in foliage measured in 1.89 kg oat forage fresh weight that was collected from 16 plots (oat plants were25 cm tall). This means that sheep ate 19.36 g soilkg^{-1} dry matter of forage, while Wilkinson et al. reported a mean intake of 101 g soilkg^{-1} total dry matter intake by sheep. Differences between these studies may be attributed to the type of forage and soil conditions.

Metals in sheep tissues

Table 2 shows that from 1.5 to 5.8% of the total metal concentrations were added through wastewater and biosolids to the soil, and from 94 to

98% of the metal contents were already present in the soil. Chromium and Pb concentrations were higher than Cd and Ni concentrations in biosolids applied at a high rate. Regarding the chemical fraction, Cr had the highest concentration in soluble and exchangeable forms (2,843 mgkg^{-1}) which indicated high availability for plant uptake. However, oat plants did uptake more Ni and Pb during the four sampling periods and were 1.44 and 1.77% of the total available soluble metal concentration.

Analysis of variance showed significant treatment effect ($P<0.01$) on Cd concentration in animal tissues (Table 3). Cd concentration in Sheep grazed in biosolids treatment plots were almost double than the control. Chromium was not detected by the chemical analysis using an atomic absorption spectrophotometer. Underwood and Suttle [22] indicated that Cr toxicity is not common and normal concentrations were below 0.1 mgCrkg^{-1}. There were not statistical differences among treatments for the heavy metal concentrations in kidney, liver and muscle ($P>0.05$).

Cd concentrations increased by 9.22% in kidney and 5.2% in liver of the sheep grazed in the treatment with 50 Mg biosolids ha^{-1} when compared with the sheep sacrificed initially (Table 3). However, Cd levels were lower than 1 mgkg^{-1} which is normal for sheep tissues [22]. Despite no significant differences observed among treatments for Ni and Pb, high values were observed in kidney, but metals concentrations were not toxic to animals (Table 4).

A comparison of the maximum metal concentrations among the three sheep initially sacrificed and the sheep grazed at the experiment is presented in Table 4. Biosolids treatment had the highest concentrations of Cd, and the maximum concentrations of Ni and Pb were observed in the fertilizer treatment. However, there were no significant differences among treatments for the three metals (Figure 6). Consistently, high concentrations of metals were found in the kidney (Table 4), and the highest concentration observed in this study was 13.3 mg Nikg^{-1}. However, toxicity level of Ni concentration in kidney is 38 mgkg^{-1} [32,21]. Also, maximum Pb levels observed were

Trace metals	Treatments			
	Control	Fertilizer	25 Mg ha^{-1}†	25 Mg ha^{-1}
	---------------------------------- mg -----------------------------------			
Cd	44	48	42	57
Cr	242	312	266	312
Ni	772	879	968	921
Pb	1613	1493	1709	1586

† Biosolid rates, dry matter basis

Table 1: Estimates of the forage Cd, Cr, Ni and Pb intake by sheep in 206 days at the Juarez Valley, Chihuahua, Mexico.

Metal (mg/kg)	Irrigation water A	Biosolids 50 Mg ha^{-1} B	Soil (initial) C	A+B+C	Available expected in soil†	Uptake oat plant‡	Uptake from available§
	-------------------------------------- g ha^{-1}---------------------------------%						
Cd	0.4	64.5	4,227	4,292	1,931	0.324	0.017
Cr	226.0	1,647.0	30,440	32,313	2,843	1.944	0.068
Ni	399.0	267.0	29,370	30,036	1,712	24.62	1.438
Pb	223.0	1,052.0	26,060	27,335	1,285	22.72	1.768

† Soluble & exchangeable fractions were 45, 8.8, 5.7, and 4.7% for Cd, Cr, Ni, Pb
‡ Based on 3.6 t ha^{-1} dry basis and 4 harvest times
§ Proportion uptaken by plant from the amount expected as available in soils († column)

Table 2: Balance of heavy metals in the system evaluated under field conditions.

Treatments	Ingested†	Kidney	Liver	Muscle
	(mg 206d^{-1})	---------------- Cadmium (mg kg^{-1}) ----------------		
Control	44.3	0.1445c‡	0.1652c	0.1449c
Fertilizer	48.3	0.2915b	0.2479b	0.2897b
25 Mg ha^{-1}	42.7	0.4194a	0.3556a	0.3972a
50 Mg ha^{-1}	56.6	0.4172a	0.3977a	0.3758a
		------------------ Nickel (mg kg^{-1}) ------------------		
Control	772	1.2481a	1.8407a	1.2392a
Fertilizer	879	3.2054a	1.4555a	1.4414a
25 Mg ha^{-1}	968	3.4106a	1.9449a	1.2809a
50 Mg ha^{-1}	921	1.2137a	1.2723a	2.3189a
		------------------- Lead (mg kg^{-1}) -------------------		
Control	1613	0.7369a	0.5424a	0.6627a
Fertilizer	1494	0.5472a	0.6882a	0.5399a
25 Mg ha^{-1}	1709	0.4828a	0.4677a	0.5421a
50 Mg ha^{-1}	1586	0.6017a	0.5724a	0.4645a

† Chromium ingested from 266 to 312 g in 206 days
‡ Row means followed by different letters are significantly different at the 0.05 probability level

Table 3: Mean concentrations of heavy metals in sheep tissues.

Metal (mg kg $^{-1}$)	Control Baseline†	Treatments			
		Control	Fertilizer	25 Mg ha^{-1}	50 Mg ha^{-1}
Cd	0.383 (kidney)	0.268 (Liver)	0.377 (kidney)	0.582 (kidney)	0.479 (kidney)
Cr	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Ni	3.571 (Liver)	4.242 (Liver)	13.318 (kidney)	7.335 (kidney)	4.049 (Meat)
Pb	1.218 (Meat)	0.991 (Meat)	1.039 (Liver)	0.911 (Meat)	0.973 (kidney)

† Three sheep sacrificed before start the experiment

Table 4: Maximum values observed for heavy metals in sheep tissues.

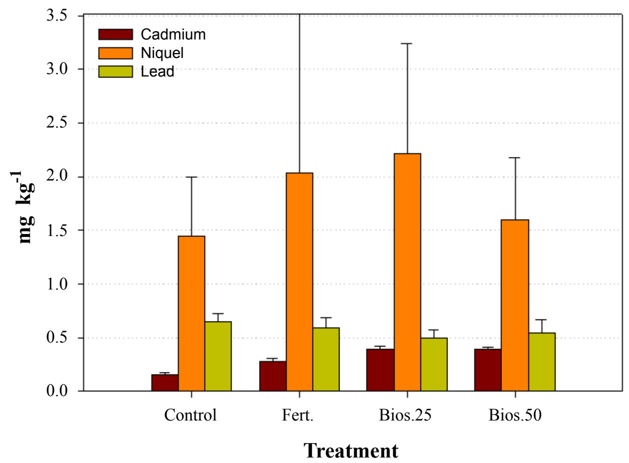

Figure 6: Trace metals content in the sheep grazed on forage oat at the Juarez Valley, Chihuahua, Mexico.

below the critical concentrations, for example, the Economic Europe Community specified levels of 2 mg Pbkg^{-1} [22].

Heavy metal concentrations in kidney, liver and muscle resulted in a normal range for all treatments. Nickel concentrations were high in all samples, and bioaccumulation was observed when comparing the initial control versus the difference in concentrations between the experimental control and the sheep grazed in the treated soil (Table 4). Increases in Ni concentrations can be attributed to the amount added to the soil and wastewater, and Ni ingested directly from the soil.

High Ni and Pb concentrations in oat forage were observed in treatment with 50 Mg biosolids ha^{-1}. For example, oat harvested in plots 6 and 10 had 41.4 and 82.9 mgNikg^{-1}, and 5.5 and 17.6 mgPbkg^{-1}. Similarly, sheep grazed in treatment with 50 Mg biosolids ha^{-1}presented higher Ni concentrations from 2.38 to 4.1 mgkg^{-1} than other treatments.

Lead concentration varied from 0.9 to 1.2 mgkg^{-1}, however the toxic level can reach at concentrations higher than 8 mg kg^{-1} [22]. The highest Pb concentration was 1.218 mg kg^{-1} in the sheep initially sacrificed before start of the experiment which indicated that no bio- accumulation of Pb in animal tissues (Table 4). Cadmium concentrations were not high, but an increase was observed in the sheep grazed in the treated soil. This suggested that longer grazing periods in soils irrigated with wastewater and treated with biosolids may represent a risk for bio-accumulation of trace metals (Table 5). In general, sheep health was good throughout the duration of this study.

The mean live weight of the sheep was not affected significantly by treatments for nine measures recorded each 15 days. Figure 7 shows the increases in weight for the total grazing period. Sheep used as control had the most weight throughout the study. On an average, mean rates were 7.4 and 6.7 g of weight gain per day in 2003 and 2004, respectively. The mean live weights of sheep were 23.36 kg at the start and 37.1 kg the end of the experiment, respectively, for the treatment with 50 Mg biosolids ha^{-1}.There is a need to conduct more studies to increase animal exposure to trace metals, and to assess metal accumulation for at least two years in soils irrigated with wastewater.

Conclusions

Heavy metal concentrations in wastewater and lime-stabilized biosolids were below critical limits of the Mexican and USEPA regulations. Maximum concentrations in wastewater observed were 165 and 259 ug L^{-1} for Cr and Ni, and in biosolids were Cr and Pb: 32.8 and 20.9 mg kg^{-1}. Soluble and exchangeable forms in biosolids were very low, but both forms in soil were high for Cd (32.3%) only.

Heavy metals concentrations in oat plants were not significantly different among treatments. There was no significant treatment effect on metal concentrations in animal tissues. Cadmium concentration increased by 9.22% in kidney and 5.2% in liver for the sheep grazed in the treatment with 50 Mg biosolids ha^{-1} when compared with the sheep sacrificed initially. Metals concentrations were in normal ranges and within the range of values reported in other studies.

The mean live weight of the sheep was not significantly affected by treatments, and the animals showed good health with no apparent problems over the period of the study. No bio-accumulation in both plant and animal tissues were observed at toxic levels, this indicated

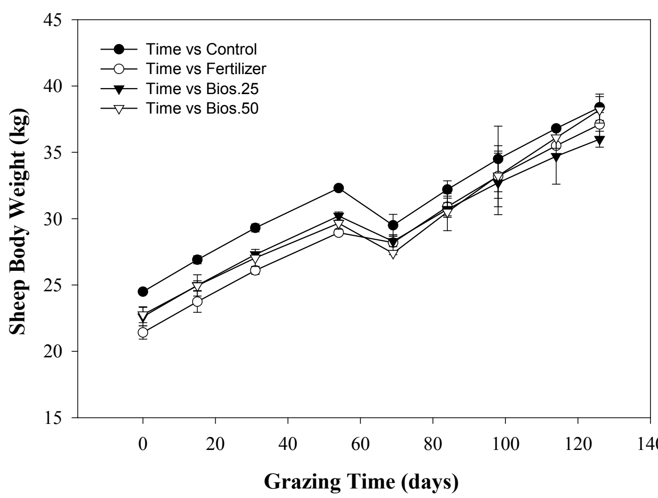

Figure 7: Sheep weight during the grazing period at the field experiment in years 2003 and 2004.

Parámeter	Series	Observations
Soil	Cr > Ni >Pb>Cd	Before apply biosolids
Biosólids	Cr >Pb> Ni >Cd	First samples
Wastewater	*Ni > Cr >Pb>Cd*	Irrigations
Forage (2003)	Pb> Ni > Cd >Cr	Oat and alfalfa
Forage (2004)	*Ni >Pb> Cd >Cr*	Oat and alfalfa
Ingested by sheep	Pb> Ni > Cr >Cd	1.5 kg forage day^{-1}
Initial sheep tissues	Ni >Pb> Cd >Cr	Kidney, liver, muscle
Final sheep tissues	*Ni >Pb> Cd >Cr*	Kidney, liver, muscle

Table 5: Series of heavy metal concentrations for the food chain including soil treated with biosolids, wastewater, oat forage, and sheep tissues at the Juarez Valley, Mexico, 2003-2004.

that there is no risk of metal contamination when consuming sheep products from animals grazed for six months. More studies are needed to assess metal accumulation and nutrient balance for at least two years in soils treated with biosolids and irrigated with wastewater. As the biosolids characteristics vary from WTP to another plant care should be taken in expanding and implementing results of this study in biosolids produced in other WTP.

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