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RESEARCH ARTICLE

Acid soil amended in contaminated conditions: Effect on cultivated lettuce (*Lactuca sativa var. longifolia*)

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ABSTRACT

Background/Aim: The waste amendment is a common practice to improve crop production on acid soils. This work aims to study the effectiveness of water potabilization plant sludge and lamb manure in acid soils remediation and the possible absorption of metals in cultivated lettuces, as well as the elements´distribution in roots and leaves after extra-contaminated irrigation. **Methods:** $Al_2(SO_4)_3$-stabilized sludge, and lamb manure was added to an acid soil collected from Huasca de Ocampo, Hidalgo State, Mexico. The sludge (L), manure (M), control soil (C), unamended (S) and amended soils (SML) were characterized, and the metals´ concentrations determined. The SML was prepared by adding 2.62 % M and 5.25% L to fill up 600 L tanks. Lettuces (*Lactuca sativa var. longifolia*) seeds were sown in S and SML, and irrigated once by metal salts solution (Na, Mg, Ca, Fe, Ni, Cu, Zn, and Cd as sulfates, 200 mg kg^{-1} each). **Results:** Lettuce germinated and grew satisfactorily in the SML, but they grew slowly in the unamended acid S, and the roots were affected. Leaves and roots were regularly analyzed along three months, and the metal concentrations showed higher values in S than the ones in SML. Pollutants were available from S, as they were present in roots, and desorbed after 40 days. The elements were present in higher concentrations in lettuce roots than in leaves, as expected. **Conclusions:** a) Acid soils can be amended with potabilization plants wastes in mining zones, but pH must be controlled to prevent the availability of metals. b) The soil texture was the principal cause of infertility due to root damage, and wastes eliminate the impairment. c) The element concentrations present in the leaves were acceptable for human nutrition.

Keywords: Acid soils amendment; lamb manure; lettuces (*Lactuca sativa var. longifolia*); metal contamination; water plant sludge

INTRODUCTION

It is well established that crop production on acid soils can be significantly risen when the soil is commonly amended with manure (Whalen et al., 2000, Gai et al., 2018) which reduces toxicity of some heavy metals, stimulating plant growth and increasing the yield of crops.

pH improves plant-availability of macronutrients while reduces the solubility of Al and Fe. Fixation of nutrients by Al is a significant impairment in acidic soils. At pH > 9, Ca and Mg fix nutrients such as P (Jones, 2012).

Chemical fertilizers tend to leach below the root zone leading to ground-water contamination, especially by nitrates (Liang et al., 2013) which can damage the soil in the long- term. Therefore, there should be alternative ecofriendly agricultural practices (Hernandez et al., 2016). Soil amendment is a recurrent practice to mitigate soil degradation, enhancing moisture retention, and rising the organic matter content.

The application of animal manure is a traditional agricultural practice for low fertility soils (Tien et al., 2019; Manojlovic et al., 2017), but the long term effects of this practice is not studied well (Gai et al., 2018). Biosolids from water treatment plants are frequently used to amend soils. This practice resolves not only the soil fertility but also, the waste accumulation of vast volumes of sludge produced. However, these deactivated biosolids are usually contaminated with metal ions and do not fit the standards for agriculture. The nutriments transportation from root to leaves in plants convey the dissolved salts through the xylem and phloem cells if they are not fixed in the root as chelates (Gupta et al., 2016)

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Uribe (2002) added biosolids (13 t ha^{-1}) as a soil amendment and achieved a 17 - 31% increase of alfalfa production than the chemical fertilization. In addition, the heavy metals concentrations in the soil and crop leaves resulted below the critical value. Hernandez-Herrera et al. (2005) applied bovine manure and wastewater sludge from a treatment plant as a soil amendment in *Sorghum vulgare pers* crop, which resulted in better production than with the chemical fertilizers. Also, the rational use of biosolids was safe and ecologically feasible. Acid soils were amended with water plant sludge, with high amounts of $CdSO_4$ and absorption studies were performed with lettuce (*Lactuca sativa var. longifolia*), tomato (*Lycopersicon esculentum Mill*), sweet corn (*Zea mays L.*), and Swiss chard (*Beta vulgaris var. cicla*) without risk to human consumption (Mahler et al., 1978).

The National Institute for Statistics and Geologic Information, (INEGI, 2014) reports that soil degradation in the Hidalgo State is severe (13,432 km^2) and all options should be considered to amend agricultural soils. The St. Thomas soil in Huasca de Ocampo, (Hidalgo State, Mexico) was chosen as the case study, whose agricultural acid soil has been used for corn crop with low production despite yearly chemical fertilization without appropriate control causing the soil degradation and acidification. Replacing it by manure and remediation plant sludge could minimize the production costs and improve the soil quality. The sludge from "El Bordo" water potabilization plant, near St. Thomas, would be an alternative to amend these soils.

The objectives of this study are (i) to characterize Los Angeles and Santo Tomas acid soils, and "El Bordo" treatment plant sludge; (ii) to determine the effect of the mixture sludge/lamb manure in the acid soil amendment; (iii) to evaluate the lettuce metal absorption and their distribution in roots and leaves, including Na, Mg, Ca, Al, Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb.

MATERIALS AND METHODS

Case study area and sample collection

Figure 1 shows the map of the case study. Its average temperature is 14 °C. A 10 x 10 m of an acid soil area in Santo Tomas was selected, and 100 kg was sampled for the experiments. This soil was cultivated with corn and chemically fertilized for years. At another site, near the dam "Los Angeles" samples were collected to be used as a control soil, for comparison.

Acid soils were collected at 20 cm from the top in two agricultural sites in the St. Thomas region at the northeast of Hidalgo State (20°, 13´, 38" NL; 98°, 38´, 4" LW, 2305 m altitude), Mexico, in the fall of 2015. The soils were Red Ferritic Luvisol, and their characteristics are displayed in Table 1.

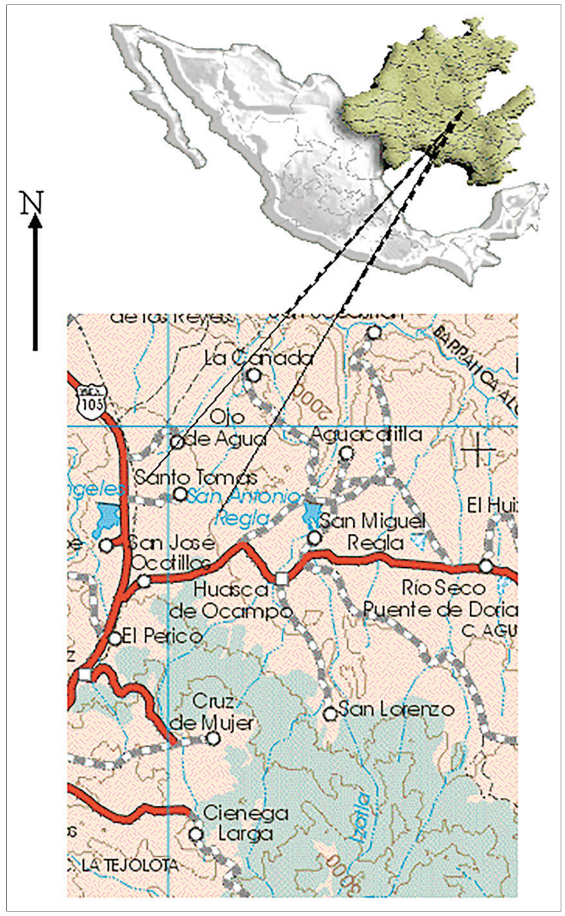

Fig 1. Santo Tomas, Huasca de Ocampo, Hidalgo State, Mexico. 20° 13´ 38" N; 98° 38´ 04" W).

Both soils were sampled following the random zigzag scheme. Twelve samples (500.0 g each) were obtained at 20 cm deep (NOM-SEMARNAT-021-2000). They were ground, mixed, and 10 mesh (1.730 mm) sieved. The bulk was quartered several times until 1 kg is left to perform the analysis. 30 kg of sludge was collected from the "El Bordo" potable water plant (20° 8´ 58" N, 98° 44´ 27" W) at 6 km from St. Thomas. The Jaramillo and Estanzuela ponds supply the water which is treated with Al_2SO_4 and filtered with sand. Sludge (60 L: 6 samples of 10 L each) was randomly collected (NOM-004-SEMARNAT-2002) during the cleaning process of the plant. The same procedure was done for lamb manure, collected in the farm "El Mercillero," Epazoyucan. (NOM-004-SEMARNAT-2002). The lambs were fed with grass. The glass containers used for biosolid samples collection were cleaned following the NOM 004 procedures with nitric acid. The physicochemical characteristics of biosolids are outlined in Table 2.

Table 1: Physical and chemical characteristics of soils, lamb manure, and sludge †

	Soil (S)	Control soil (CS)	Manure (M)	Sludge (L)	Amended Soil (SML)
pH H_20	5.83	5.63	9.30	5.67	6.77
pH KCl	4.58	4.89	8.72	5.21	6.35
% C	0.56	1.14	24.21	4.62	2.53
% N	0.06	0.03	1.73	1.05	0.11
% OM	1.15	1.96	82.18	3.55	7.35
CEC	5.8	5.9	39.6	21.1	28.2
Available cations concentrations (mg kg^{-1})					
Na^+	0.29	0.22	-	19.0	24.71
K^+	9.91	8.42	-	17.0	14.85
Mg^{2+}	0.51	0.51	-	12.0	50.93
Ca2+	1.00	0.99	-	46.0	62.95
Color Munsell					
Dry	5 YR 4/4 Brown-reddish	5 YR 4/4 Brown-reddish	-	5 Y 7/3 Pale-Yellow	5 YR 4/4 Brown-reddish
Wet	10 R 3/2 Dark red	10 R 3/2 Dark red	-	10YR4/3 Brown	10 R 3/2 Dark red
Density					
Apparent Density	1.11	1.07	-	0.74	1.09
Real Density	2.39	2.37	-	2.09	2.23
Total porosity	53.55	54.85	-	35.40	51.12
Texture (%)					
Sand	20	22	-	-	19
Silt	18	20	-	-	20
Clay	62	58	-	-	60

†Values are the means of at least 4 determinations. Nutrient analyses are expressed on a dry weight basis. NOM-SEMARNAT-021-2000.

Soils, biosolids, and their mixtures analysis

Analytical-grade chemicals, TraceMetal™ grade acids, and distilled–deionized water (20 dS m^{-1}) were used in this study. Soil texture analyses were performed using the international pipette method (Gee and Bauder, 1986). Soils and biosolid pH were determined with a Corning pHmeter 340 on 1:25 soil/water slurries after 30 min equilibration. The Organic Matter was determined by the modified Walkley and Black method. Organic C and N were measured using a Perkin – Elmer series II 2400 C and N analyzer. Determination of % N-NH_4 was done by Nessler (Jeong et al., 2013). Colors were defined based on the Munsell Soil Chart, 1975 (Viscarra et al., 2006). Apparent density measured by the paraffin method and real density by a Pycnometer. Porosity = Apparent Density/Real Density) x 100. The Cation exchange Capacity (CEC) was measured using $BaCl_2$ as an index ion (Hendershot and Duquette, 1986). Total metal contents were measured using AAS Perkin Elmer 2100 after decomposition of samples by Method 3051A microwave-assisted acid digestion of sediments, sludges, soils, and oils (EPA, 2007). Available Na^+, K^+, $Mg^{+,}$ and Ca^+ were determined by extraction with $NH_4(CH_3COO)$ 1N, pH 7 and further analyzed by AAS Perkin Elmer 2100. Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solids, Total Solids data in Table 1 were performed following the methods described in NOM 004 (2002).

Table 2: Physico-chemical characteristics of the sludge†

Parameter	El Bordo
BOD (mg/l)	4052.70
COD (mg/l)	5403.60
Suspended Solids (mg/l)	154.60
Total Solids (mg/l)	197.30
S. D. T (mg/l)	35.30
% Grease	2.66
% Moisture	99.37
% NH4	0.01

† Values are the means of at least 3 determinations. NOM-SEMARNAT-004-2002.

Statistics

Data were evaluated statistically, and comparisons allowed with ANOVA by SPSS 12.0 software. Table 3 shows the metal determination data with statistical parameters in soils, biosolids, and mixtures. Table 3 shows the final value's data of metal concentrations and the standard deviations in lettuces (leaves and roots). The standard deviation and % error of analytical measurements of elements demonstrate the reliability of obtained values.

Experimental

The experiment was carried out in four 600 L round tanks (0.88 m diameter x 0.91m high) with a side drain at the bottommost for leaching. Gravel was previously washed with HNO_3 10% and water. 250 kg clean gravel was placed

Table 3: Total metal concentrations of soils and amendments#, with±SD and %Error data†. (mg . kg^{-1})

	Al	Ca	Cd	Cr	Cu	Fe	Mg	Mn	Na	Ni	P	Pb	Zn
L (Sludge)	132.5±0.40 (0.30)	54.75±0.5 (0.9)	0.69±0.05 (7.25)	1.70±0.10 (5.88)	1.85±0.08 (4.32)	242.05±0.50 (0.21)	2.50±0.05 (2.00)	12.88±0.30 (2.33)	72.75±0.61 (0.81)	3.70±0.01 (0.27)	1.09±0.10 (9.17)	1.42±0.03 (2.07)	1.29±0.03 (2.33)
M (Manure)	4.57±0.06 (1.31)	84.34±0.10 (0.12)	0.12±0.02 (16.67)	0.42±0.02 (4.76)	0.16±0.01 (6.25)	33.28±0.10 (0.30)	84.16±0.07 (0.08)	1.87±0.06 (3.19)	16.43±0.08 (0.49)	2.26±0.03 (1.33)	0.69±0.02 (2.90)	0.79±0.03 (3.80)	1.49±0.09 (6.04)
C (Control Soil)	150.06±0.40 (0.27)	21.50±0.06 (0.28)	0.04±0.01 (25.00)	0.19±0.02 (10.53)	0.03±0.002 (6.67)	38.59±0.06 (0.16)	1.50±0.06 (4.00)	1.14±0.01 (0.88)	2.66±0.06 (2.00)	0.46±0.05 (10.86)	1.26±0.02 (1.59)	0.89±0.02 (2.25)	0.18±0.02 (11.11)
S (St Thomas)	156.2±0.24 (0.15)	23.80±0.30 (1.26)	0.51±0.02 (3.92)	2.15±0.05 (2.33)	0.30±0.02 (6.67)	70.61±0.20 (0.54)	16.13±0.22 (1.36)	11.24±0.06 (5.08)	30.98±0.05 (2.02)	4.55±0.07 (1.54)	1.31±0.10 (7.63)	3.91±0.03 (1.52)	1.73±0.09 (5.20)
SM (Manure amended soil)	141.1±0.60 (0.43)	38.12±0.30 (0.79)	0.51±0.01 (1.96)	0.49±0.02 (4.08)	0.28±0.007 (2.50)	64.16±0.20 (0.31)	14.58±0.21 (1.44)	2.67±0.20 (7.49)	28.07±0.10 (0.36)	4.10±0.10 (2.44)	1.29±0.02 (1.55)	0.65±0.02 (1.21)	0.54±0.02 (3.70)
SML (Manure and sludge amended soil)	144.4±0.90 (0.62)	41.20±0.10 (0.97)	0.53±0.02 (3.77)	1.90±0.10 (5.26)	0.72±0.02 (2.78)	71.85±0.07 (0.09)	20.30±0.10 (0.49)	2.82±0.10 (3.57)	34.90±0.20 (0.57)	4.18±0.04 (0.96)	1.30±0.03 (2.31)	0.72±0.20 (12.05)	1.47±0.07 (4.76)
SMLMet0 (initial, contaminated with metal solution)	143.6±0.84 (0.58)	66.40±0.40 (0.60)	2.10±0.20 (9.52)	1.97±0.05 (2.54)	2.40±0.03 (1.25)	116.0±0.10 (0.11)	42.50±0.40 (0.94)	4.19±0.20 (4.77)	41.00±0.20 (0.49)	5.30±0.20 (3.77)	1.18±0.16 (13.56)	0.85±0.10 (6.06)	2.15±0.002 (0.80)
(SMLMet95) fin (final, after lettuce harvest)	143.0±0.20 (0.14)	4.40±0.20 (4.55)	0.11±0.01 (9.09)	0.17±0.02 (11.76)	0.03±0.004 (13.33)	19.95±0.08 (0.40)	2.93±0.05 (1.71)	1.39±0.03 (2.16)	6.40±0.51 (7.97)	0.30±0.03 (10.0)	1.10±0.02 (1.81)	0.08±0.01 (12.50)	0.23±0.02 (8.70)
Mexican Standards*		2-10	<3		>0.2	>4.5	0.5-3	>1		<100	5.5-11	<100	>1
European Standards**			1-3	50-150	50-140					30-75		50-300	150-300

† SD: Standard Deviation, E: % Error. #Values are the means of at least 3 determinations. * NOM-021-RECNAT. 2000 **(Kabata, 2001).

at the bottom of the tank, forming a foundation of 26 cm. The amended soil was placed over the gravel until a total high of 0.33 m, so it remains 32 cm free to the top of the tank. Two tanks were filled with the control soil (C) and the other two, with the mixture soil/manure/sludge (SML). The mixture was prepared as follows. 100 kg (92.1%) of air-dried sieved soil (<1.730 mm) was mixed with fresh lamb manure 2.85 kg (2.62%) and 5.7 kg sludge (5.25%). The physical and chemical properties of the soil, lamb manure, and sludge are outlined in Table 1.

Four replicates determinations of unamended control acid soil (C), studied acid soil (S), manure (M), sludge (L) and the amended soil (SML) was performed in each tank.

Lettuce seeds (*Lactuca sativa var. longifolia*) were sown in a 60 grid (4 seeds each) of 6 x 10 pattern on the surface of each tank and sprinkled irrigated daily with 0.3 L distillate water during the three months experiment. At day 5, the soil was contaminated once with 1 L of an aqueous solution of 200 mg kg^{-1} of sulfate salts of Na^{+}, Mg^{2+}, Ca^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} (analytical grade, Aldrich). The effect of lamb manure and sludge on soil fertility was determined by measuring the time of seed germination and the seedling growth. The lettuces grew until 8 cm and then, samples were taken every two weeks. The chemical analyses (NMX-B231-1990) were achieved by microwave destructively sampling (0.200 g each, 105° - 180°C, 10 mL HNO_3, 20 min, in a (CEM Mars-X, 1200 W). The lettuce roots and leaves were collected every two weeks, and three replicate samples analyzed at 15, 47, 55, 59, 79 days (in S) and 15, 25, 35, 57, 79, and 95 days (in contaminated SML_{Met}) after the planting seeds, considered the beginning of the experiment. Metals were determinate from the extract of the destructed samples.

The SML were also analyzed in the contaminated soil SML_{Met0}, and the end of the experiment (79 and 95 days, respectively). The final soil SML_{Met95} was analyzed after the lettuce harvest.

RESULTS AND DISCUSSION

Biosolids and soils

Tables 1 and 2 display the general characteristics of soils, biosolids, and amended soil studied in this work. Biosolids were characterized by the NOM 004 (2002) procedures. Metal concentrations in soils, amendments, and its mixtures are shown in Table 3 and represented in Figures 2 and 3.

The sludge (L) showed high Fe and some Al occurrence. Hidalgo State is rich in minerals, so, the water treated in "El Bordo" is coming from the mining zone in the mountains, explaining the high values of Fe. Al in the sludge is mainly due to the use of $Al_2(SO_4)_3$ in the flocculation process. The sludge shows a low amount of organic matter (See Table 2).

Sludge was odorless due to its neutral pH. The sediment solids (S.S) are low and resulted in low levels of organic matter and N% and C%. Humidity is high. They were rich in Al, Fe, Na, Ca y Mg and in significant concentrations Mn, Ni, Cu, Cr, and Pb. Due to Cd, Mn, Ni, and Pb concentrations, sludge did not fulfill the requirements of the Mexican Norm 004 (2002) for the use of biosolids in agriculture. Unfortunately, Mexican standards (NOM-004-SEMARNAT-2002) concerns only six elements, excluding Na, Mg, Al, Ca, Mn, Fe, and P.

On the other hand, lamb manure has a very high percentage of organic matter, as it was expected (See Table 2). Lamb manure showed low content of Ni and Cd, with a significantly high presence of Mn, and fit the standards criteria NOM 004 (2002) for suitable biosolids. Mg and Ca are presented in the highest concentrations. See Table 3 and Figure 3. The presence of Mn, Ni, and Cd in manure could be due to the water used to feed the lambs.

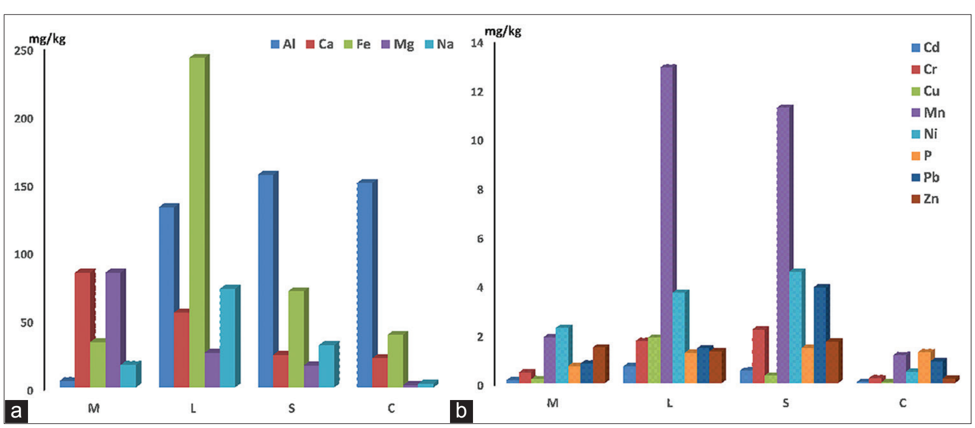

Fig 2. (a,b) Element concentrations in M: Lamb Manure. L: Sludge. Soil (S), Control Soil (C).

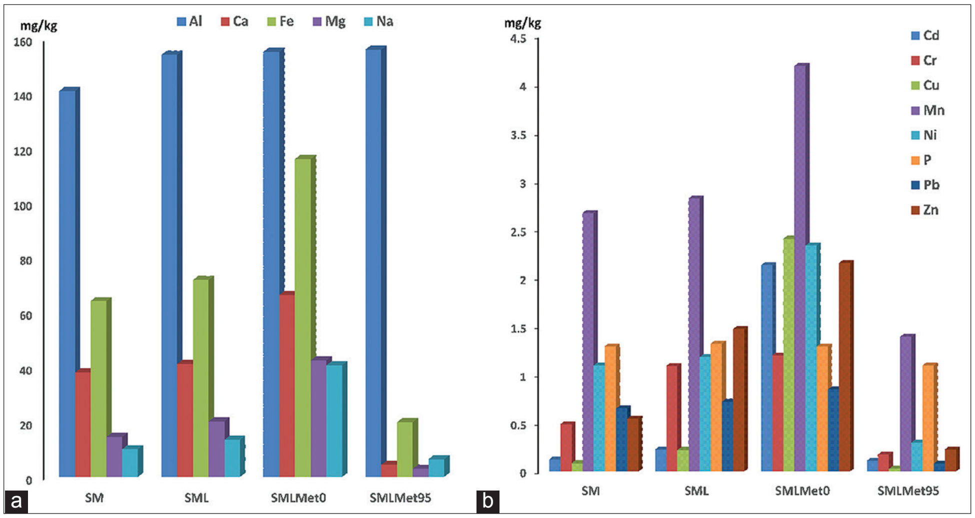

Fig 3. (a,b) Element concentrations in Amended soil (SM) and (SML). Amended soil irrigated with metal contaminated water SMLMet0 at the initial (t=15 days) and SMLMet95 (t=95 days) final time.

Soils

Table 1 shows that the control soil texture differs from that of Santo Thomas unamended soil (S). The control soil had a deficient cation exchange capacity and no available elements. It showed an unfavorable texture, as the clay fraction was predominant, and a lousy texture with a more significant clay fraction than the control soil (C).

Both acid soils have low % of organic matter, and the texture of control soil is the worst. Urea is currently used as fertilizer, in Santo Tomas, and it drops the soil pH, leading to available toxic Al species and lowering the productivity of the crop (Honorato, 2000).

There is stormy weather in the summer in this region. That is why; the ion leaching boots the formation of acid soils (Bickelhaupt and White 1982; Brady and Weil 2007, Rupiasish et Vidyasagar, 2009). The essential ions (Ca^{2+}, Mg^{2+}, K+ y Na^{+},) are leached and replaced by cationic interchange with Fe^{2+}, Fe^{3+}, Mn^{2+} and occasionally Al^{3+}, whose dissolution is favored in the acidic soils (pH < 5.5) andbecome available to plants (Samac and Tesfaye 2003).

Figure 2 illustrates the comparison of metal concentrations between control agriculture soil (C) and Santo Tomas soil (S) as well as the lamb manure (M) and "El Bordo" sludge (L). S presents more metals than C. Both soils fit the European Community (Kabatas & Pendham, 2001), and Mexican standards recommended values (NOM 004, 2002).

Among the main elements, Al and Fe show the highest concentrations in both soils, which are rich in metals due to the mining nature of the region. Among the minor, Mn presents the highest levels, which is an essential nutrient for crops, because it is involved with the enzymes related to growing metabolic process and tolerant to diseases.

Amended soil

Table 1 displays the characteristics of soils, biosolids, and mixtures. After amendment with biosolids, Santo Tomas agriculture soil quality improved in many parameters, and fertility would recover. S was moderate acidic with a low content of organic matter and cation exchange capacity. Due to the 58 - 62% of clay, the soils have good physical and chemical activity. pH increased from 5.83 to 6.77, as well as the CEC, from 5.8 to 28.2 $cmol^{+}kg^{-1}$, allowing the cations are more available due to the amendment. The sludge improves the soil texture and the CEC. Also, organic matter (OM) raises from 1.15 to 7.35%, the C does from 0.56 to 2.53%, and N from 0,06 to 0.11% due to the addition of manure.

Metal concentrations of soils, amendments, and its mixtures are shown in Table 3 and Figures 2 and 3. There were no significant differences in metal concentrations between S and amended soils (SM and SML), with high Al and Fe in all mixtures. After amendment (SML) the soil is still with high Fe, Na, Mg, Ca concentrations, caused by the presence of these elements in the sludge.

Manure provides Ca, Mg, Na, Ni, and Zn as well as organic matter. The presence of Ca is highly significative (P > 0.05) and explains the lowering of acidic characteristics of the soil. Exchangeable Ca (23.8 to 41.2 mg kg^{-1}) were significantly higher in amended soil. It was reported that animal manure raises the pH value (Whalen et al., 2000, Gai et al. 2018) and therefore, lowers the availability and toxicity effect of metal present in the soils (Tang et al., 2007). Lambs were fed with grass and CaO processed corn ("nixtamal") that explains the high levels of Ca and Mg in the manure.

Sludge conveys Al, Fe, Cu, Mn, and Ni, but no organic matter. See SM and SML in Figure 3. Al is present at high levels in both acid soils (S and C). The Al concentrations from sludge are due to the flocculation process in "El Bordo" is carried out with Al_2SO_4. Al concentration remains high before and after the amendment. Mn is prevalent among the minority elements, but its concentration does not surpass the critical toxic level reported in soils (Fernando and Lynch, 2015).

P concentrations do not change significantly with the amendment. The sludge is high in Na, due to the $NaCO_3$ added in the potabilization process, so the salinity raises in the amended SML.

The main improvement in SML mixture was the pH raising, best texture, enhanced organic matter, and cation capacity interchange.

Lettuce

The lettuce seeds germinated in the unamended C tanks but stopped growing up to 3 cm in the 25th day and died, due to the lousy texture of C, which does not allow water infiltration. In the amended soil tanks, the lettuces grew satisfactory, reaching 40 cm high, as shown in Figure 3.

Al in C is high and available due to the acid condition and damages the main branches of the root system in plants (Vega-Corea, 2005; Kochian et al., 2004; Hue & Licudine, 1999). The inadequate texture of C and its low cation exchange capacity (Table 2) prevented the germination of lettuces. Amended soil improved those parameters and lettuces germinated despite further contamination of the soil when it was sprayed with 200 mg kg^{-1} metals solutions. Al was not available during the lettuce crop experiments, due to the raised pH, and the lettuce roots were well formed. It was confirmed because Al levels in the soil did not change during the experiments with lettuce crops.

Metal bioaccumulation in leaves and roots of lettuces planted in amended plus contaminated soil are shown in Figures 4 and 5, respectively. The chemical analysis shows the variations of metal concentrations in leaves and roots throughout the growth of the plants, but the values are near the limits of the experimental determination. It has been reported that lettuces do not absorb mineral content even with overfertilization and high variability data at such low concentrations (Slamic and Jug, 2017).

Metal in lettuce: Leaves

Lettuce seeds were harvested as indicators for the metal distribution in the plant, coming from contaminated soil. *Lactuca sativa var. longifolia* was chosen due to previous reports as absorbing plant, used for soil bioremediation. (Hernandez et al. 2016, Liu et al. 2018).

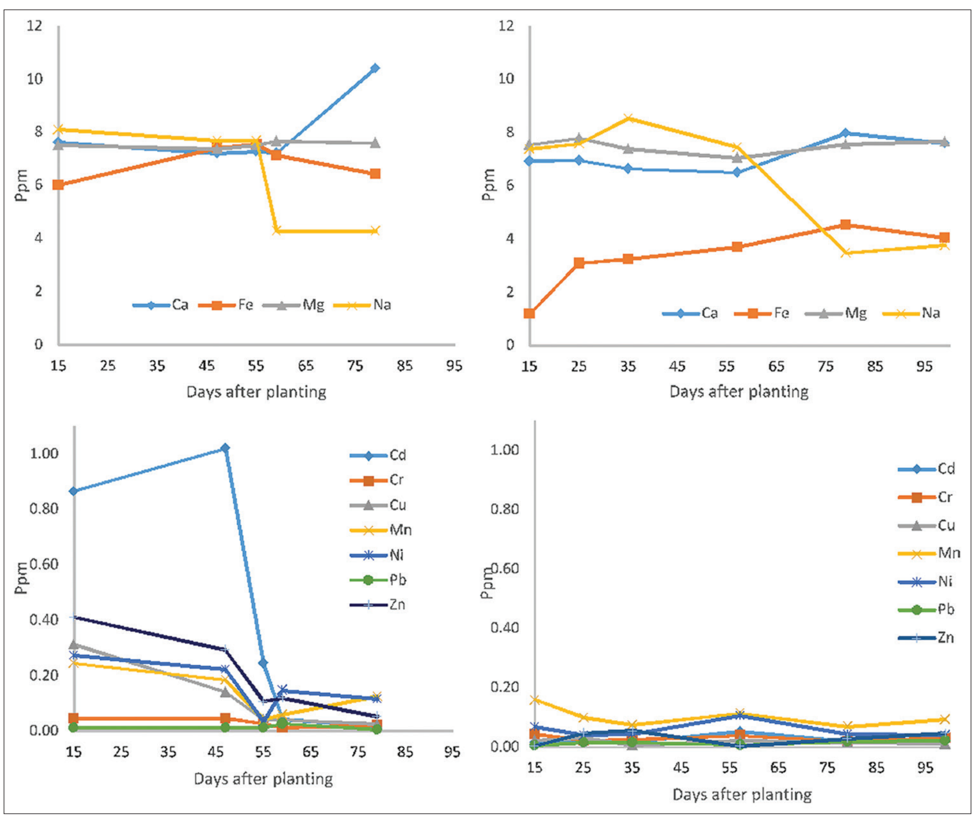

Fig 4. Metals content vs. time in lettuce leaves.

The importance of soil texture in the lettuces´ germination and growth in the C was confirmed. The amended soil showed good fertility, and lettuces grew satisfactory as it is shown in Figure 6. Most of the lettuce seeds did not germinate in the control soil C. The plasticity and adhesiveness of soil lead to water accumulation, and no oxygen reached the roots of lettuce. When the soil dried out, it became hard and impermeable, and lettuces died.

Macroelements Na, Mg, Ca, and Fe

In acid soils, Fe is toxic in concentrations higher than 25 mg kg^{-1} (Samac y Tesfaye, 2003). The amended soil presents 71.85 mg kg^{-1} Fe, and it was harmless to lettuces which germinated and grew satisfactorily.

Mg is determinant to the synthesis of chlorophyll, and Fe is a catalyst for this process. It plays a significant role with the enzymes and compounds involved in the light energy transmission in photosynthesis. Deficiency of Mg and Fe can cause loss of characteristic green color and chlorosis. Leaves become yellow and have deficient texture (Hernandez et al., 2016). None of those signs were observed in the experiment.

Figure 4 illustrates the variation of element concentrations in lettuce leaves along 95 days in control and amended

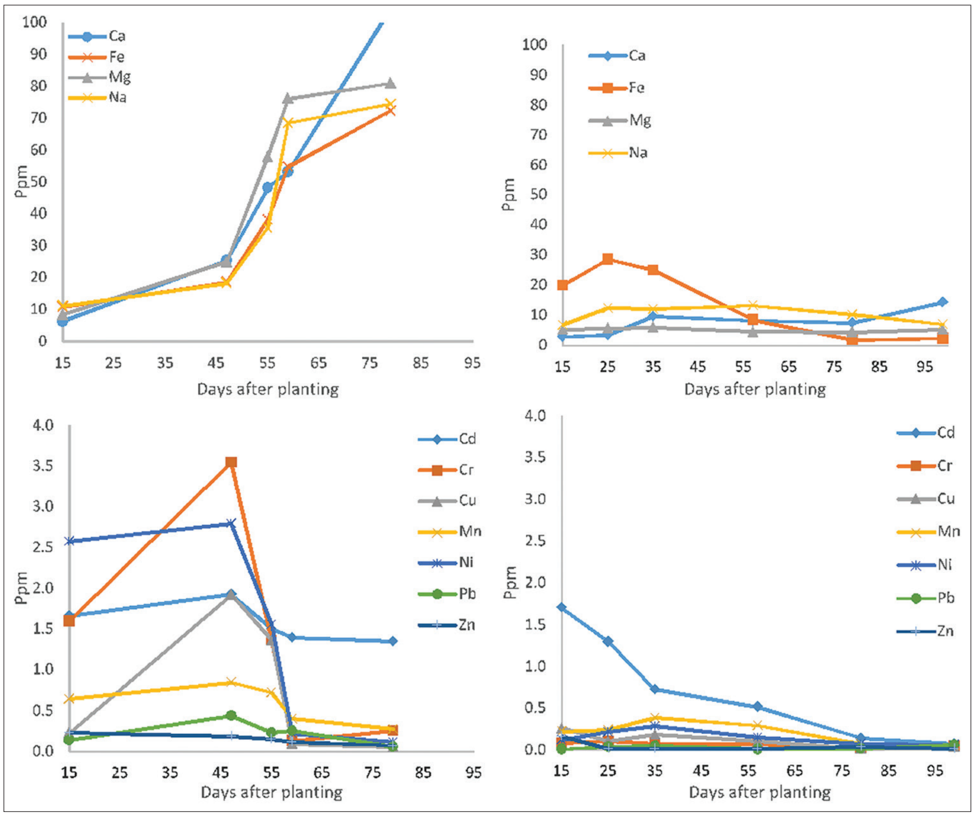

Fig 5. Metals content vs. time in lettuce roots.

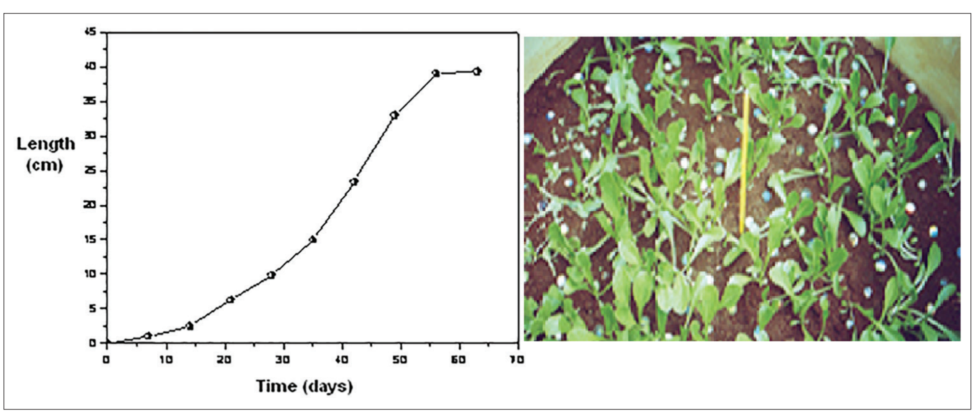

Fig 6. Lettuces growing evolution in the amended soil.

soil. Na, Mg, Ca, and Fe showed the highest values in both soils, where Na drops sharply after 59 days. Ca and Fe concentrations rises slightly up to 10 and 4, respectively. Mg is almost constant along with the experiment in 8 mg kg^{-1}.

Leaves showed higher metal absorption during the first 42 days when the growing metabolism predominates, and micronutrients are needed. The lettuce in C soil showed higher concentrations than S soil (Cd, Zn, Ni, Cu, Mn) but all are <1 mg kg^{-1}. After 45 days all decrease below 0.2 mg kg^{-1}. Microelements are in detected at the detection limits of the method, and they show a wide dispersion, so, the results are taken as indicative as a tendency.

Microelements. Cu, Zn, and Cd

Zn has a vital role in the lettuce enzymatic system, which regulates the growing process. It is a crucial element for the hormonal equilibrium, especially for the activity of the growth hormone auxin. This metal remains almost unchanged for 96 days and then falls drastically.

Mn was present in low concentrations (0.19 - 0.12 mg kg^{-1}) and did not change significantly during the experiment. Mn is a cofactor for dehydrogenases, oxidases, and carboxylases. The leaves do not absorb heavy metals. Levels are below 0.2 mg kg^{-1} in the amended soil. The element present in the leaves were in acceptable concentrations for human nutrition.

Metal in lettuce: Roots

The microelements in roots showed the highest concentrations in C than SML. All metals´ concentrations rise significantly except Zn (from 0.2 - 2.6 mg kg^{-1} to 0.4- 3.5 after 47 days, dropped sharply and ended below 0.6 mg kg^{-1}. Cd remained at 1.4 mg kg^{-1}, is the highest quantity of the elements absorbed in roots. So, the acid conditions favored the absorption and translocation of the elements. In contrast, the roots showed slight absorption in SML, and all elements are <0.1 mg kg^{-1} at the end of the experiment. Cd behaves differently, starting with 1.7 mg kg^{-1} and dropping along the days.

In general, the elements were more concentrated in roots than in leaves, as expected. Cd concentrations remained absorbed in roots, only in acidic conditions, showing phytoremediation potential of lettuces at pH 5.63 or less.

The macro elements in roots were Ca, Mg, Na, and Fe, as in leaves. Figure 5 shows the concentration variations in roots during the experiment timeline. Roots showed the highest concentrations in the C experiment, rising all concentrations significantly (from 6-11 mg kg^{-1} to 72 and 104 mg kg^{-1}, as the acid conditions favored the absorption and translocation of the elements. In contrast, roots lettuces in the SML amended soil showed a very slightly absorption. Na and Mg remain almost constant (5 and 7 mg kg^{-1}, Ca rises from 3 to 14 mg kg^{-1} and Fe behaves similarly as in leaves, rising at the beginning and dropping after 55 days to 2 mg kg^{-1}.

Roots were not atrophied and progressed satisfactorily. So, Al did not accumulate, and the absorption of P and Ca was suitable (Samac, 2003).

Metal absorption by lettuce in contaminated soils

Figure 3 shows the contaminated amended soil at the beginning (t=0) and etnd (t= 95 days) of the experiments. The final soil SML_{Met95} drop most of the metal concentration, mainly in case of Na, Mg, Ca, Fe, Cr Mn, Ni, Cu, Cd, and Pb. The metal concentrations were diminished more than ten folds. Moreover, none was fixed in the roots, so lettuces did not behave as a phytoremediation option.

On the other hand, Al, P, and Zn remain unchangeable. They remained fixed in the soil and was not lixiviated, possible due to pH 6.77, risen after amendment. Therefore, Al availability turns off. That is why roots and leaves were in good shape in the SML cultivated lettuce. Al is the principal cause of rachitic lettuces (Kochian et al., 2004). It agrees with the fact that Al presents the same concentration in the soil along with the lettuces crops experiment (See Table 3).

CONCLUSIONS

The studied acid soils presented deficient texture, low organic matter content, and cation exchangeable capacity. They were not contaminated with toxic metals and presented high concentrations of Al and Fe. Lamb manure and water potabilization plant sludge fit the Mn, Ni, Zn and Pb limits of Mexican standards. The amended acid soil with 2.62 % lamb manure and 5.25% sludge had improved soil fertility by better texture, cation capacity interchange organic matter, raised pH, and reduced the availability of metals for lettuce absorption. Despite the metal addition in the first irrigation, no toxic metals were found in leaves, and low concentrations of toxic metals were present in roots, concluding that the contaminants were not absorbed, and the leaves were acceptable for human nutrition. Studies should be done for other crops to determine the proportions of sludge and lamb manure. This paper presents a solution for the disposal of the water treatment plant sludge, the remediation of acid soil, and at the same time, an alternative of local agriculture improvement.

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Authors' contribution

ADRG and GPBU performed the experiments and chemical analysis. FPG made the statistics and results´ analysis. JRVI and AJGM participated since the initial project and discussed the results. ADRG and EMOS made the whole analysis, graphics, and wrote the paper.

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