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ScienceDirect
Journal of Hazardous Materials 146 (2007) 91–97
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Chromium fractionation in semi-arid soils amended with chromium and tannery sludge

M. Barajas-Aceves *, J. Corona-Hernández, R. Rodríguez-Vázquez

Department of Biotechnology and Bioengineering, CINVESTAV-IPN, Av. IPN 2508, San Pedro Zacatenco C.P., 07300 México, D.F., Mexico

Received 4 May 2006; received in revised form 24 October 2006; accepted 1 December 2006
Available online 5 December 2006

Abstract

We studied Cr fractionation in three semi-arid soils (cultivated, under-the-canopy, and outside-the-canopy soils). The soils were amended with: Cr^{3+}, Cr^{6+}, tannery sludge, Cr^{3+} + tannery sludge, and Cr^{6+} + tannery sludge and all soils were incubated for 30 and 120 days at 25 °C. The Cr in three semi-arid soils was fractionated using sequential extraction (Tessier scheme). Data of Cr fractionation were used to find the correlations with microbial activities determined in previous work. The microbial activities: CO_2–C evolved, dehydrogenase activity and nitrification were determined in the same soils amended with the same treatments. Tannery sludge was added at 0.0125 g g^{-1} soil and Cr^{3+} or Cr^{6+} at 250 μg g^{-1} soils. After 120 days of incubation, higher values of concentration of Cr were found in the residual fraction in the three soils amended with all the treatments, except cultivated and outside-the-canopy soils amended with Cr^{6+} + tannery. The non-residual fraction tended to decrease with time except in cultivated and outside-the-canopy soils amended with Cr^{6+} + tannery sludge. CO_2–C evolved was significantly correlated ($p < 0.05$ and $p < 0.001$) with fractions bound to: Mn oxides, Fe oxides organic matter at 30 and 120 days of incubation (from $r = 0.827$ to 0.979). Dehydrogenase activity was correlated with fractions bound to Fe oxides and bound to organic matter, and nitrification with fraction bound to organic matter at 30 days of incubation ($r = 0.874$, 0.959, and 0.803, respectively). These results suggest that even in a sparingly available Cr fraction in semi-arid soils has effect on microbial activities.

Keywords: Tannery sludge; Fractionation of Cr; Semi-arid soils; Tessier scheme; Microbial activity

1. Introduction

Large concentration of N- and C-rich organic residues are contained in the sludge from leather processing in the town of Leon, Guanajuato, Mexico, that produces up to 64,320 tonnes sludge $year^{-1}$ (CIATEC, Internal Report). The sludge also contains large amounts of Cr and toxic organic compounds that have serious problems to the environment and humans. The use of tannery sludge as organic fertilizer to semi-arid soils at north of Guanajuato state may provide valuable nutrients to pioneering vegetation [1]. The application of this organic residual will also increase organic matter content, cation exchange capacity and preventing further degradation of soil structure and thus erosion.

Tannery sludge contains both trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) chromium: Cr^{6+} is much more reactive, toxic, and shows a higher mobility in the soil than Cr^{3+}. The speciation of Cr has significant importance in the time risk of Cr contamination in soils and toxic wastes. Thus, Cr^{6+} can be reduced to Cr^{3+} in soils by redox reactions with aqueous inorganic species, reactions with organic compounds such as carbohydrates and proteins, or reduction by soil humic substances [2–4]. Chromium(III) complexed with soluble organic ligands will remain in the soil solution [5]. In addition to decreased Cr^{3+} adsorption, added organic matter may also facilitate the oxidation of Cr^{3+} to Cr^{6+}. Soils that are low in organic matter and high in manganese(IV) oxides deserve special attention. This type of soils might be able to oxidize Cr despite the fact that this process was generally found to be very slow [6,7]. There are no data available regarding the chemical forms of Cr in semi-arid soils amended with Cr^{6+}, Cr^{3+}, and tannery sludge. This information is critically important for assessing mobility of heavy metals.

* Corresponding author. Tel.: +55 5061 3316; fax: +55 5061 3313.
E-mail address: mbarajas@cinvestav.mx (M. Barajas-Aceves).

0304-3894/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.jhazmat.2006.12.001

Heavy metal pollution does not only result in adverse effects on plant quality and yield, but also cause changes in the size, composition and activity of microbial community in the soil [8]. Numerous studies have demonstrated the adverse effect of different heavy metals on soil microbial biomass and activity [9–12].

The maintenance of soil fertility depends on the biomass and activity of soil microorganisms, which are of fundamental importance in the biological cycles of almost all the major plant nutrients [13]. The microorganisms also are involved in forming the structure of a soil [14]. A number of soluble microbiological parameters have been suggested as possible indicators of soil environmental quality [15]. Thus, soil respiration, enzyme activities such as dehydrogenase activity and nitrification, can give information on the presence of viable microorganisms as well as on the intensity and on the kind and duration of the effect of pollutants on the metabolic activity of soils.

Evaluation of total metal content may be useful as a gross index of contamination but it provides minimal insight into the potential mobility of the metals under field conditions [16].

The scheme of Tessier et al. [17] is widely used. The application of sequential extraction is still subject to much controversy. The main problems of sequential extraction procedure are: non-selectively of the extracts and trace elements redistribution among phases during extraction [18]. Despite these restrictions, sequential extraction procedure has proved to be useful in the field of environmental analytical chemistry [19].

Few reports have been published to determine relationships between microbial properties and trace metal forms in soils [20].

The aims of this work were to determine the Cr fractionation in semi-arid soils amended with Cr^{3+} and Cr^{6+}, both alone and mixed with tannery sludge. Data of Cr fractionation were used to determine the correlation between chemical forms of Cr and microbial activity assessed in previous studies. The microbial activities (CO_2–C evolved, dehydrohenase activity and N mineralization) affected by Cr amendment with the same treatments in the same semi-arid soils were used in the correlationship. The information generated from this study may be useful in assessing the beneficial use of tannery sludge in reforesting semi-arid soils.

2. Materials and methods

2.1. Experimental site

The soil was sampled from three sites: mesquite (*Prosopis laeviginata*) dominant vegetation (Dolores Hidalgo, Guanajuato, Mx) under the canopy and outside the canopy of mesquite and one site cultivated with maize (*Zea mays*) for 20 years.

2.2. Soil sampling

The soil was collected from 0 to 5 cm layer where it shows the highest organic matter content and a deeper layer showed a compact structure soil. The sampling took place under the canopy of 4 isolated mesquite trees and 1–2 m from the stem in 4 perpendicular directions selected at random.

The second sampling took place in the same perpendicular direction at the distance of 6–8 m from the stem that is outside the canopy cover of the mesquite tree. The cultivated soil was sampled from the 0 to 30 cm layer of the agricultural land at Dolores Hidalgo, Gto. The soil was bulked; all the stones, visible roots and fauna removed, sieved to less than 2 mm and stored at 5 °C until used.

2.3. Tannery sludge

Tannery sludge produced during leather manufacturing was sampled from a tannery from Leon (Guanajuato, Mexico). The sludge contained large quantities of hair, soluble proteins and fatty flashings from processing the skin to hide, and sulphide, lime, chromium-sulphate, salts, dyes, acid and leather trimmings from processing the hide to leather. The chemical characterization is shown in Table 1. The tannery sludge was air-dried before used for the experimental aerobic incubation.

2.4. Aerobic incubation

Before the experiment, the soils were adjusted to 40% of their total water holding capacity (WHC) and conditioned at 25 °C for 7 days in 60 kg sealed drums (4 kg per drum) containing 200 ml distilled water at the bottom to avoid desiccation and containing a beaker with 100 ml of 1 M NaOH solution to trap evolved CO_2. Sub-samples of 40 g of soil were placed in 110 ml glass bottle which were subsequently put into 1 l jar which contained 10 ml H_2O to avoid desiccation and a vessel with 20 ml of 1 M NaOH solution to trap CO_2–C evolved.

The following treatments with three replications were applied to the soil: control (without any amendment) Cr^{3+}, Cr^{6+}, tannery sludge, Cr^{3+} + tannery sludge, Cr^{6+} + tannery sludge. Cr^{3+} (Cr_2O_3) and Cr^{6+} ($K_2Cr_2O_7$) were added to soil at a rate of 250 μg g^{-1} soil. The dose was selected as a criteria near to

Table 1
Characteristics of tannery sludge from Leon, Guanajuato, México

pH	8.09
Moisture (g kg^{-1})	880
Organic C (g kg^{-1})	257.8
Total N (g kg^{-1})	18.7
Total P (g kg^{-1})	7.5

	Zn (89 mg kg^{-1})	Cu (14 mg kg^{-1})	Ni (1.3 mg kg^{-1})	Cd (4 mg kg^{-1})	Cr (1663 mg kg^{-1})	Pb (15 mg kg^{-1})
EU upper limits	4000	17500	400	40	ND	12000

EU upper limits value for sewage sludge used in agriculture; ND, not given.

the value of the maximum upper limits of 300 mg kg^{-1} for acceptable utilization of waste and bioproducts in agriculture as established by the US Environmental Protection Agency, Part 503 [21].

The amount of tannery sludge added was 0.0125 g g^{-1} soil equivalent to 345% of the requirement region recommended dose of N for maize crop (i.e. 260 kg N ha^{-1}). The jars were sealed with air-tight plastic lids and incubated at 25 °C for 120 days. After 0, 30 and 120 days, the vessel with 20 ml of 1 M NaOH solution was removed, resealed and stored until analysis of CO_2. At the same intervals, the soil was removed for analysis of NO_3^- by shaking for 30 min with 100 ml of 0.5 M K_2SO_4 solution and filtering through Whatman No. 42 paper. The extractants were stored at −20 °C until analysis. After 15, 40 and 90 days all the jars were opened, the vessel with 1 M NaOH solution replaced with fresh NaOH solution and the jars were resealed and further incubated at 25 °C.

2.5. Soil microbial activities and nitrification

Soil dehydrogenase activity was measured using a modification of the method of Casida [22]. The CO_2 trapped in 1 M NaOH solution was measured titrimetrically with a standard HCl solution. The concentration of NO_3^- in the extracts was determined by colorimetric method [23].

2.6. Soil metal analysis

Total metal concentrations in tannery sludge were determined using absorption atomic spectrometry after digestion (digiprep TM digestion system) using Aqua Regia [24]. Total Zn, Cr, Cu and Ni were measured in flame atomic absorption spectrometry, Pb and Cd were determined by absorption atomic spectrometry fitted with a graphite furnace Avanta M System 300, GF 3000 S/N 10288.

Sequential extraction was utilized for partitioning Cr in soil and sludge amended soils into six operationally defined fractions, exchangeable (I), bound to carbonates (II), bound to Mn oxides (III), bound to Fe oxides (IV), bound to organic matter (V) and residues (VI) according to the procedure described by Tessier et al. [17]. After 0, 30 and 120 days of incubation, the level of Cr in the six fractions (I–VI) was analysed with Atomic Absorption Spectrometry as described above.

All measurements are the mean of triplicate determinations of three separate jars and are given on an oven-dry basis (105 °C, 24 h). All the statistical analysis was performed using a statistical analysis package SAS [25], version 9.0.

3. Results and discussion

3.1. Fractionation of chromium

The speciation patterns in exchangeable, bound to carbonates, bound to metal oxides (Fe and Mn) and bound to organic and residual forms were determined and their sums normalized to 100% in the three soils: cultivated (C), under the canopy (B), and outside the canopy (F) at 0, 30, and 120 days incubation

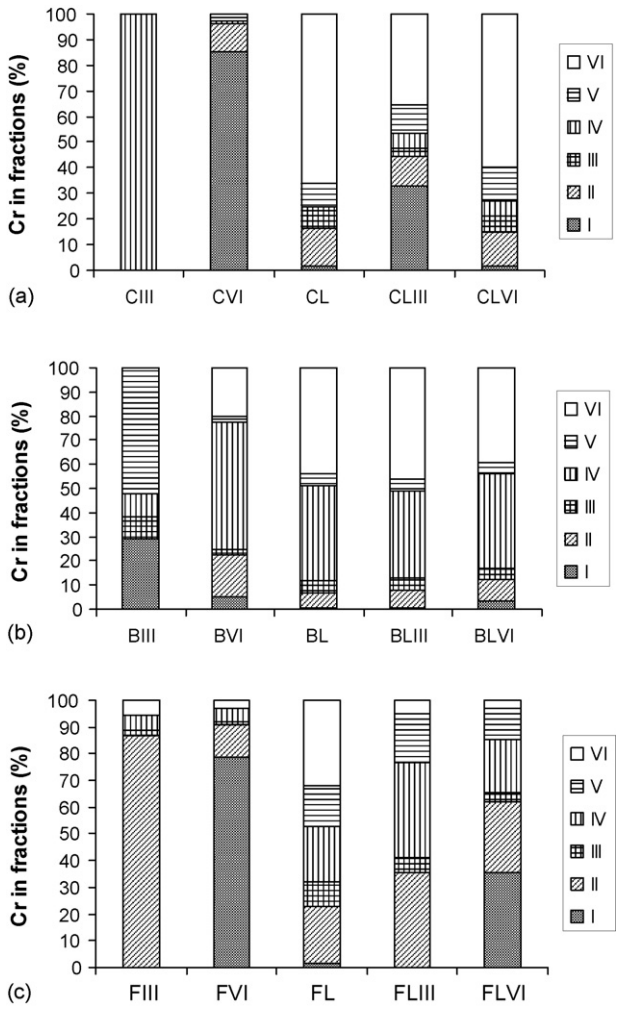

Fig. 1. Fractionation of chromium between various phases (%) in: (a) cultivated, (b) under the canopy and (c) outside the canopy soils from Dolores Hidalgo, Mexico, incubated at 25 °C for 0 days. Treatments were: soil amended with Cr^{3+} (III), Cr^{6+} (VI) tannery sludge (L), tannery sludge and Cr^{3+} (LIII), and tannery sludge plus Cr^{6+} (LVI).

are shown in Figs. 1–3, respectively (except cultivated soil at 30 days incubation).

The values of exchangeable Cr in the three soils amended with Cr^{6+} alone or Cr^{6+} plus tannery sludge were higher in Cr^{6+} alone than Cr^{6+} plus tannery sludge in the three soils at 0, 30 and 120 days incubation (Figs. 1–3). It seems that Cr^{6+} were absorbed most strongly onto organic matter of tannery sludge and the presence of organic matter, Cr^{6+} will be reduced to Cr^{3+} [26].

The values of every Cr fractionation in the three soils treated with tannery sludge alone or tannery sludge plus Cr^{6+} or Cr^{3} were similar. The distribution patterns of Cr in the three soils with those treatments were found as follows:

- At 0 days incubation:
  - *Cultivated*: residual > carbonates > organic > Mn oxides > Fe oxides > exchangeable.
  - *Under the canopy*: residual > Fe oxides > carbonates ≫ Mn oxides > organic > exchangeable.

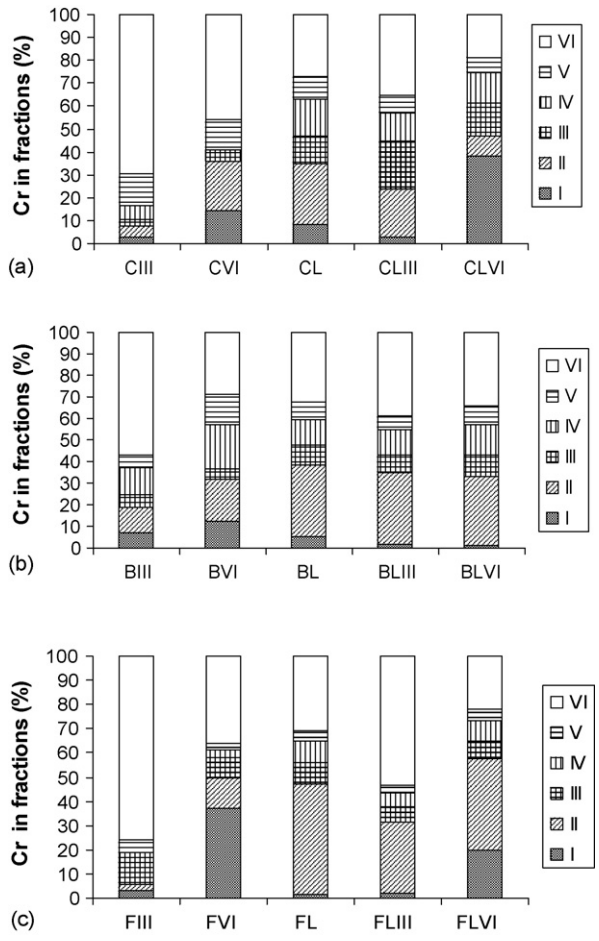

Fig. 2. Fractionation of chromium between various phases (%) in: (a) cultivated (C), (b) under the canopy (B) and (c) outside the canopy soils (F) from Dolores Hidalgo, Mexico, incubated at 25 °C for 30 days. Treatments were: soil amended with Cr^{3+} (III), Cr^{6+} (VI), tannery sludge (L), tannery sludge and Cr^{3+} (LIII) and tannery sludge plus Cr^{6+} (LVI).

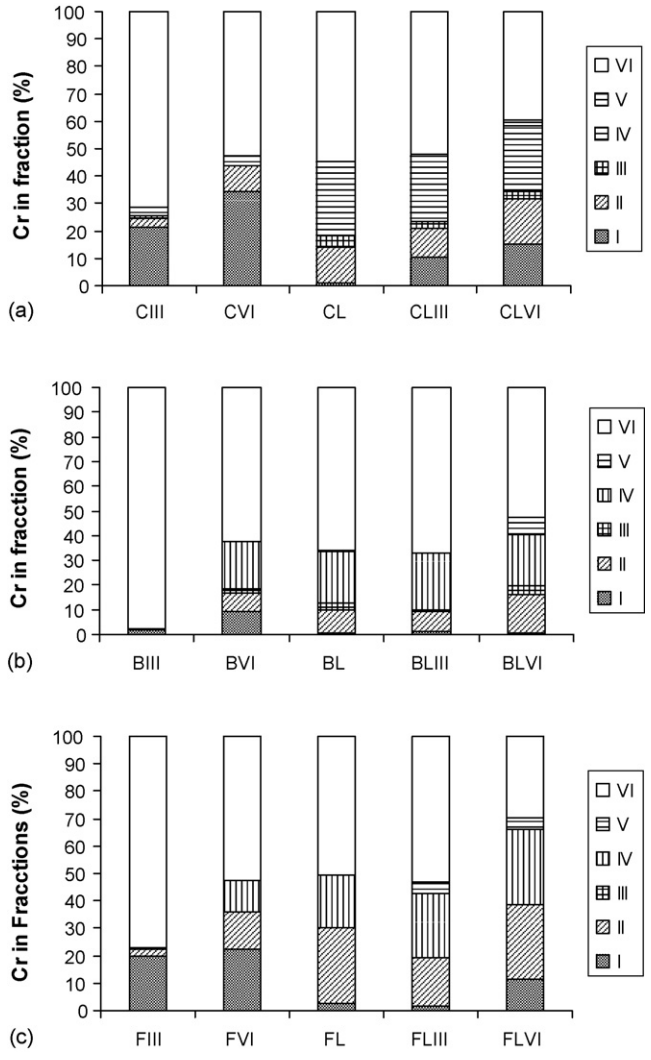

Fig. 3. Fractionation of chromium between various phases (%) in: (a) cultivated (C), (b) under the canopy (B) and (c) outside the canopy soils (F) from Dolores Hidalgo, Mexico, incubated at 25 °C for 120 days. Treatments were: soil amended with Cr^{3+} (III), Cr^{6+} (VI) tannery sludge (L), tannery sludge and Cr^{3+} (LIII) and tannery sludge plus Cr^{6+} (LVI).

  - *Outside the canopy*: carbonates > Fe oxides > organic > Mn oxides > residual > exchangeable.
- At 30 days incubation:
  - *Cultivated*: residual > carbonates > Fe oxides > M oxides > organic > exchangeable.
  - *Under the canopy*: residual > carbonates > Fe oxides > M oxides > organic > exchangeable.
  - *Outside the canopy*: carbonates > residual > Fe oxides > Mn oxides > organic > exchangeable.
- At 120 days incubation:
  - *Cultivated*: residual > Fe oxides > carbonates > exchangeable > organic > Mn oxides.
  - *Under the canopy*: residual > Fe oxides > carbonates > Mn oxides > organic > exchangeable.
  - *Outside the canopy*: residual > Fe oxides > carbonates > exchangeable > Mn oxides > organic.

The difference in distribution pattern can be attributed to possible mobilization of the metals. These results suggest that Cr^{6+} or Cr^{3} or total Cr from tannery sludge, were absorbed in the same compartment in the same soil and the dominant fraction of Cr seems to depend on the physicochemical characteristics of the soil. Thus, outside the canopy soil which had low organic C, the dominant trace element sink could be carbonates due to the high rate of percolation water and carbonates are easily dissolved and leached out [27]. Before 30 days incubation, the presence of carbonates was the most important mechanism for regulating the behaviour of Cr [27] in outside-the-canopy soils. Xian [28] reported that metals associated with carbonates would be susceptible to changes in pH, also are easily made soluble. After 120 days incubation, the pattern of Cr distribution in outside-the-canopy soils reached the same pattern as it was observed on cultivated and under-the-canopy soils.

The greater percentage of Cr in the residual fraction at 120 days incubation (Table 2) (40–67%) probably reflects the grater tendency of Cr to become unavailable once it was in the soil. These results agree with those of Lu et al. [29] who reported that soluble metals added were transformed from easily extractable fraction to more stable fraction.

Table 2
CO_2 production rate, dehydrogenase activity, and inhibition of NO_3^- concentration in cultivated, under- and outside-the-canopy soils from Dolores Hidalgo, Mexico, incubated at 25 °C for 30 and 120 days

Treatment	% Inhibition (30 days)	% Inhibition (120 days)
CO_2 production rate		
Cultivated + Cr^{3+}	14.40 dc	0
Cultivated + Cr^{6+}	71.48 b	58.08 a
Cultivated + sludge + C^{6+}	8.69 de	0
Under-the-canopy + Cr^{6+}	21.49 c	25.12 b
Under-the-canopy + sludge + Cr^{6+}	7.57 de	14.71 c
Outside-the-canopy + C^{6+}	98.48 a	0
Outside-the-canopy + sludge + Cr^{6+}	22.11 c	0
Dehydrogenase activity		
Cultivated + Cr^{3+}	18.72 dc	56.94 b
Cultivated + Cr^{6+}	51.60 ba	40.02 c
Cultivated + sludge + C^{6+}	43.46 b	43.13 c
Cultivated + sludge + C^{3+}	0	26.51 d
Under-the-canopy + Cr^{6+}	15.39 d	15.06 e
Under-the-canopy + sludge + Cr^{6+}	29.93 c	31.48 d
Outside-the-canopy + C^{6+}	61.26 a	83.63 a
Outside-the-canopy + sludge + Cr^{6+}	49.61 ba	0
NO_3^- concentration		
Cultivated + Cr^{6+}	11.93 e	27.95 c
Cultivated + sludge + C^{6+}	74.51 a	52.05 b
Under-the-canopy + Cr^{6+}	52.82 dc	0
Outside-the-canopy + C^{6+}	69.64 ba	82.91 a
Outside-the-canopy + sludge + Cr^{6+}	62.40 bc	85.69 a
Outside-the-canopy + sludge + Cr^{3+}	43.80 d	29.48 c

Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$, according to the Duncan's test.

Among the non-residual fraction the prominent fraction varies from soil to soil. Most of the Cr in non-residual fraction was associated to bound Fe oxides which agrees with the reports published elsewhere [30,31]. Hydrous oxide of Fe is the most important compound in the sorption of trace metallic pollutants [27]. These results suggest that disposal of tannery sludge on semi-arid soils might not have a hazardous effect on the semi-arid ecosystem after 3 months of application. However, further studies are required to evaluate the availability of Cr to plants in soils amended with tannery sludge and the rate of sludge application.

In general, the trends for the fractionation of Cr in the three soils are to increase in the residual fraction from 30 to 120 days of incubation and to decrease in the non-residual fractions (Table 2) such as exchangeable, bound to carbonates, bound to Mn and Fe, and bound to organic matter.

The active organic sites in outside-the-canopy soils might be blocked by Fe(III) (oxy) hydroxides present in the soil. Thus, the presence of Fe(III) (oxy) hydroxides could decrease the reductive capacity of the soil, inhibiting the reduction of Cr^{6+} or absorption of Cr^{3+} [32–34]. Another explanation might be that Cr^{6+} complexed with fulvic acid from tannery sludge [35–37].

The low inorganic matter and high concentration of Mn in cultivated and outside-the-canopy soils (0.60, 0.27 g C kg^{-1} and 274, 185 mg Mn kg^{-1} soil, respectively) might also be an important factor to consider when examining the chemical behaviour of Cr in interactions with the minerals in the soils [33]. Finally, all these interactions are of importance for the mobility and speciation of metals in the soils [38–40].

Table 3
Fractionation of chromium (mg kg^{-1}) in semi-arid soils from Dolores Hidalgo, México, amended with Cr^{3+}, Cr^{6+} and/or tannery sludge, incubated at 25 °C for 30 and 120 days

	Time incubation			
	30 Days		120 Days	
	Residual treatment	Non-residual treatment	Residual treatment	Non-residual treatment
Cultivated				
Cr^{3+}	16.3 e	7.2 j	33.6 ef	13.4 j
Cr^{6+}	13.1 e	15.5 i	50.8 b	45.4 c
Tannery sludge	14.5 e	38.8 g	35.7 ed	29.6 f
Tannery sludge + Cr^{3+}	16.7 e	31.0 h	35.0 def	32.2 e
Tannery sludge + Cr^{6+}	15.3 e	66.0 c	42.0 c	64.2 b
Under the canopy				
Cr^{3+}	5.4 f	4.1 j	41.4 c	0.90 l
Cr^{6+}	12.7 e	31.2 h	27.7 gh	16.8 i
Tannery sludge	26.0 d	53.6 d	50.1 b	25.7 g
Tannery sludge + Cr^{3+}	31.2 c	49.1 e	66.4 a	33.0 e
Tannery sludge + Cr^{6+}	38.4 b	74.4 b	42.2 c	38.0 d
Outside the canopy				
Cr^{3+}	22.0 d	7.0 j	39.7 cd	12.0 k
Cr^{6+}	25.0 d	44.2 f	26.5 h	24.1 h
Tannery sludge	23.4 d	52.2 d	33.0 efg	32.4 e
Tannery sludge + Cr^{3+}	45.1 a	39.1 g	29.7 fgh	26.1 g
Tannery sludge + Cr^{6+}	22.0 d	77.6 a	27.6 gh	65.5 a

Residual = fraction VI; non-residual = sum of fractions I–V. Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$, according to the Duncan's test.

Table 4
Correlation coefficients of fractionation of Cr and CO_2–C evolved, dehydrogenase activity, and NO_3^- concentration in semi-arid soils from Dolores Hidalgo, Mexico, incubated at 25 °C for 30 and 120 days

Fractionation					
I	II	III	IV	V	VI
CO_2–C evolved (30 days)					
0.165	0.687	0.827**	0.863**	0.568	0.548
CO_2–C evolved (120 days)					
−0.749	0.460	0.979**	0.965**	0.879*	0.065
Dehydrogenase activity (30 days)					
−0.492	0.502	0.414	0.874**	0.959**	0.464
Dehydrogenase activity (120 days)					
−0.613	0.249	0.673	0.628	0.440	−0.078
NO_3^- (30 days)					
−0.241	−0.332	−0.525	0.508	0.803*	−0.481
NO_3^- (120 days)					
0.246	0.663	0.303	0.646	0.346	0.187

Significant at $^{*}p<0.05$ and $^{**}p<0.001$; fractions: I (exchangeable), II (bound to carbonates), III (bound to Mn oxides), IV (bound to Fe oxides), V (bound to organic matter), VI (residues).

3.2. Relationship between microbial activities and Cr in different fractions

In a previous experiment, the three semi-arid soils (cultivated, under and outside the canopy soils) were amended with Cr^{6+}, Cr^{3+}, Cr^{6+} plus tannery sludge, Cr^{3+} plus tannery sludge, and tannery sludge alone with the same doses (0.0125 g tannery sludge g^{-1} soil and 250 μg Cr^{3+} or Cr^{6+} g^{-1} soils) in order to determine the effect of all the treatments on CO_2 production rate, dehydrogenase activity and nitrification.

The results of Table 3 show that the addition of Cr^{6+} plus tannery sludge decreased the % inhibition of CO_2 and dehydrogenase activity compared to the Cr^{6+}-amended soils, except in under-the-canopy soils for dehydrogenase activity. However, the addition of tannery sludge to cultivated soils amended with Cr^{6+} increased the % inhibition of NO_3^- concentration. There is a clear trend to decrease for % inhibition of CO_2 in the soils treated with Cr^{6+} or Cr^{6+} plus tannery sludge throughout the incubation time, but there are a not clear trend for the NO_3^- concentration and dehydrogenase activity the three soils with the same treatments (Table 3).

The relationships between microbial activities and Cr in the six different fractions were calculated using data from treatments that show inhibitions on CO_2–C evolved, dehydrogenase activity, and NO_3^- concentrations in the three soils together.

There was a highly significant ($p<0.001$) correlation between CO_2–C evolved and fraction IV (reducible fraction Fe oxides) and fraction III (reducible Mn oxides) at 30 and 120 days incubation and fraction V (organic) ($p<0.05$) at 120 days incubation in the three (Table 4). The significant ($p<0.001$) correlation between dehydrogenase activity and fractions was found for fractions V and IV at 30 days and between NO_3^- and fraction V ($p<0.5$) at 30 days incubation (Table 4). The results for % inhibition and the residual and non-residual data discussed above might suggest that the bioavailability of the metal not only depends on its concentration but it is also affected by the characteristics of the tannery sludge and soils components (such as oxides, Fe, Mn, or quality of organic matter) to which it is sorbed [3], and this will have an influence on the interaction between Cr and the biota [41].

Several attempts to quantitatively predict bioavailability and toxicity from speciation data have been unsuccessful [42]. However, in spite of the lack of information available, fractionation of Cr could give a more precise prediction of Cr bioavailability and/or inhibition of microbial activities than measurements of total Cr concentration alone.

4. Conclusions

The results of the present study indicate that after amending three semi-arid soils with Cr^{6+}, Cr^{3+}, Cr^{6+} plus tannery sludge, Cr^{3+} plus tannery sludge, or tannery sludge alone, the level of total Cr increased in the more resistant fraction (less soluble form) and further increased over time. The opposite trend occurred with the non-residual fraction, which tended to decrease with time in the three soils (except for the cultivated soils treated with Cr^{6+}, Cr^{3+}, Cr^{3+} plus tannery sludge and outside-the-canopy soils treated with Cr^{3+} alone). These results suggest that the possibility of Cr leaching or availability to plants in these semi-arid soils should be minimal.

The correlations between the amount of microbial activity affected by the treatments and the fractional phases were significant in fractions bound to Mn oxide, bound to Fe oxide, and bound to organic matter for CO_2–C evolved and dehydrogenase activity, and only fraction bound to organic matter for NO_3^- concentration. These results suggest that even in a sparingly available Cr fraction has effect on soil microbial activity.

The Cr fractionation assessment may be useful to examining the main chemical association form of Cr in sludge and amended soils, which affects the soil biological processes.

Further studies are necessary to evaluate the effects of different fractions of Cr on soil microbial health in natural environment experiments where more parameters are involved, and to investigate Cr cycling and the effects of long dried seasons.

Acknowledgments

This work was financially supported by Semarnat-Conacyt, 2002-COI-0184, Mexico. The authors thank J. López Luna for technical help.

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