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Potential Cr oxidation and metal interaction in bush bean plants grown on tannery sludge amended soil

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ABSTRACT

A study was conducted to predict the potential Cr^{3+}oxidation, heavy metal availability and accumulation in bush bean plants grown on a culture soil amended with tannery sludge. Tannery sludge amendments (0, 0.77, 1.54, 3.08 and 6.16 g tannery sludge/kg soil) were characterized and the main heavy metals identified (Cr, Mn, Fe, K, and Zn) later on singly extracted with EDTA for availability assessment. Total oxidable Cr^{3+} determination showed high tendency of Cr to be oxidized but it was prevented by neutral alkaline soil pH, despite the reactive Mn presence. Different patterns of metal availability were observed and available Cr increased with tannery sludge amendments, suggesting organic complexation as the main mobility mechanism for Cr uptake by plants. In bush bean plants metals were mainly accumulated in the roots, diminishing in the upper part with minimal translocation to pods and seeds. Important correlations were observed between metals and soil physico–chemical properties and also noticeable synergistic and antagonistic metal interactions in vegetable tissues were recorded.

KEYWORDS

Phaseolus vulgaris;
Heavy metal;
Availability;
Accumulation;
PCA.

INTRODUCTION

Leather processing in Guanajuato, Mexico, produces up to 64,320 tonnes of sludge per year. The sludge contains valuable nutrients for crops, but also large amounts of Cr and toxic organic compounds, exerting serious problems to the environment and humans[1], since both, hexavalent (Cr^{6+}) and trivalent (Cr^{3+}) chromium have been found in tannery waste. Cr^{6+} is much more reactive, toxic, and shows higher mobility in soil than Cr^{3+}. Cr^{6+} can be reduced to Cr^{3+} in soils by organic compounds such as carbohydrates, proteins and humic substances[2], remaining the organically complexed Cr^{3+} in the soil solution[3]. In alkaline soils

Cr is mostly present as insoluble Cr $(OH)_3$, which prevents Cr leaching into the groundwater or its uptake by plants. However, in more alkaline soil solutions (pH 7–10), precipitation of Cr^{3+} competes efficiently with organic complexation[4]. Soils that are low in organic matter and high in manganese (IV) oxides might be able to oxidize Cr[5]. Additionally, soil organic matter mineralization might mediate Cr^{3+} relocation and oxidation to Cr^{6+} in high tannery sludge amended soil[3,6]. Accordingly, determination of the total metal content of soil samples is not sufficient to evaluate its mobility[7]. But procedures employing a single extractant provide a suitable method to determine the potential availability of metals for plant uptake[8]. Indeed, metals extracted with EDTA have shown good correlation with plant uptake in calcareous soils[9]. Thus, this study was conducted in an agricultural tannery sludge amended soil to predict metal availability, potential oxidation of Cr^{3+} by Mn oxides, and the effect of metal accumulation and interaction on the fully–grown bush bean *(Phaseolus vulgaris)* cultivars.

MATERIALS AND METHODS

Soil, tannery sludge and test seeds

A culture sandy soil was collected at Dolores Hidalgo, Guanajuato, México (latitude 21º 13' 08" N, longitude 100º 49' 25" W). The soil was air–dried at room temperature and sieved through a 2–mm mesh. Dry tannery sludge was collected from a leather industry in León Guanajuato, México. Soil and tannery sludge were physico–chemically characterized. Seeds of bush bean (Flor de Mayo variety Bajío) were provided by INIFAP–México. Seeds were selected for uniformity and damaged ones were discarded.

Experimental setup

Experiments were conducted as described by López–Luna et al[10]. Briefly, pots without drainage holes (11.5 cm diameter) were filled with 1kg amendments containing 0, 0.77, 1.54, 3.08 and 6.16 g tannery sludge/kg soil to rend approximately 0, 50, 100, 200 and 400 mg Cr/kg soil. The seeds were disinfected in 0.1% $HgCl_2$ for 2 min, after which they were washed three times with deionized water. Four seeds were sown in each pot at a depth of 5 cm, adding previously 200 ml of distilled water to each pot. Deionized water was added as necessary to maintain the soil surface moisture throughout the test. Thirty days after sowing, each pot was complementary fertilized with 300 kg/ha ammonia sulfate (20.5 % N) and 87 kg/ha triple calcium superphosphate (46% P_2O_5). The experiment (80 pots) was conducted in a greenhouse in a completely randomized design. There were four replications and the plants were harvested at 100 days after sowing for quantifying metal accumulation.

Total metal determination

Representative samples of tannery sludge amendments and sandy soil (control soil) were digested with HCl:HNO_3 2:1 (v/v). Three replicates of each sample were evaluated. All metal samples (Cr, Mn, Fe, K and Zn) were analyzed by atomic absorption spectrometry AAS (GBC–Avanta) using the 7000A EPA–Method[11].

Hexavalent and trivalent chromium determination

Cr^{6+} determination in tannery sludge and tannery sludge amended soil was conducted by alkaline digestion using the 3060 EPA–Method[12]. Hexavalent Cr was colorimetrically determined by the 7696A EPA–Method[13]. Cr^{3+} was determined by the difference between total Cr and Cr^{6+}.

Metal availability determination

For available metal extraction, 5 g of soil in 25 ml of EDTA 0.05 mol/l (pH 7 with NH_4OH) were mechanically shaken for 1 h. Three sample replicates were determined by AAS as described before.

Total oxidable trivalent chromium

For total oxidable Cr^{3+}, 1 g sample was extracted with 40 ml of commercial bleach (6% or NaClO 0.7 mol/l) adjusted to pH 9.5 with HCl. Tubes were vigorously mixed and then placed 20 min in a water boiling bath. Samples were vigorously mixed again and centrifuged to obtain the clear extract[14]. Total oxidable Cr^{3+} from filtered samples, was AAS determined.

Reactive manganese

For reactive Mn, 1 g sample was extracted with 40 ml of 0.1 mol/l hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) in HNO_3 0.01 mol/l. Samples were mechanically shaken 30 min and then centrifuged[15]. Reactive Mn from filtered liquid supernatant was AAS determined.

Metal determination in plant tissues

Plants were uprooted and thoroughly washed with distilled water to remove the adhered soil. Next, the samples (separated into roots, shoots, pods and seed fruits) were oven–dried at 70ºC for 48 h. Then milled and weighed, and the vegetable material digested with HCl:HNO_3 (2:1; v/v) in a microwave–oven. Metals (Cr, Mn, Fe, K and Zn) from four replicates were AAS determined.

Statistical analysis

Analyses of variance (ANOVA) and LSD tests were conducted for total metal content assessment. For metals from EDTA extraction, total oxidable Cr^{3+} and reactive Mn, ANOVA and Tukey's multiple comparison tests were used to determine significant differences among treatments. Analyses of variance (GLM) and means comparisons (LSD) were conducted to identify significant differences of metal accumulation in vegetal tissues. To establish the relationships between metals extracted with EDTA, total oxidable Cr^{3+}, reactive Mn and metal accumulation in plants, a Principal Component Analysis (PCA) was conducted using a correlation matrix. Statistical analyses were performed with the SAS System Software ver. 9.0[16] at $P<0.05$.

RESULTS AND DISCUSSION

Soil, tannery sludge and tannery sludge amendments physico–chemical properties

The soil analysis in dry basis revealed the following characteristics: pH 7.61, electrical conductivity (EC) 240 μS/cm, CEC 137.5 $mmol_+$/ kg, total C 10.80 g/kg, total N 0.80 g/kg, extractable P 0.015 g/kg, total Cr 8.70 mg/kg, sand 88%, silt 7% and clay 5%. It was found a neutral alkaline sandy soil that in fact affected metal behavior being explained ahead. Sludge characteristics were: pH 8.65, total C 76.20 g/kg, total N 7.60 g/kg, extractable P 0.017 g/kg, total Cr 65 016 mg/kg, Cr^{+6} 6 mg/kg, meaning that Cr in tannery sludge is almost trivalent Cr[10]. C, N and P content in amendments was not significantly different from control soil, since little amounts of sludge were added to soil because of the high total Cr concentration in tannery sludge. Conversely, pH and EC increased with tannery sludge amendments: 0 g/kg (pH 7.61, EC 240 μS/cm), 0.77 g/kg (pH 7.84, EC 363 μS/cm), 1.54 g/kg (pH 7.85, EC 460 μS/cm), 3.08 g/kg (pH 7.88, EC 596 μS/cm) and 6.16 g/kg (pH 7.91, EC 1036 μS/cm) bringing about accentuated neutral alkaline conditions.

Total and available metal content in tannery sludge amended soil

Total Cr increased with tannery sludge amendments (TABLE 1). Cr^{6+} was not longer detected, thus total

TABLE 1 : Total and EDTA available metal content in tannery sludge amended soil.

Metals (mg/kg)	Tannery sludge addition (g/kg soil)				
	0 (control)	**0.77**	**1.54**	**3.08**	**6.16**
Total					
Cr	8.70±0.50a	53.42±5.68b	94.17±3.07c	210.58±30.14d	432.817±15.39e
Mn	186.63±15.76a	210.10 ±18.27ab	202.34±13.65ab	215.97±12.19b	211.22±3.16ab
Fe	9423.75±652.20a	8758.33±229.38a	8198.75±1013.59a	8053.75±1730.74a	9782.92±240.90a
K	1615.00±207.89a	1291.17±16.50b	1722.67±99.15a	2017.17±5.13c	1680.50±26.01a
Zn	30.47±1.60a	30.15±0.23a	31.35±2.35a	29.65±2.58a	29.76±1.00a
Available					
Cr	0.56±0.11a	9.29±0.06 b	18.64±1.23c	31.52±1.29d	57.28±5.10e
Mn	7.35±0.65a	6.95±0.09a	6.69±0.46a	6.08±0.39a	6.71± 1.27a
Fe	62.11±19.44a	56.47±17.41a	56.69±10.35a	57.37±19.88a	88.16±11.81a
K	28.11±3.71a	35.73±22.23a	23.52±9.92a	27.37±3.39a	44.78±21.33a
Zn	2.56±0.23a	2.64±0.075a	2.62±0.09a	2.91±0.290a	3.47±0.55a

Values are means of three replicates ± standard deviation. Means with the same letter are not significantly different. *P<0.05* LSD test for total metal. *P<0.05* Tukey's test for available metal.

metal content was assumed as Cr^{3+}. Tannery sludge amendments were also found to contain important concentrations of Mn, Fe, K and Zn, although without significant differences among treatments. EDTA available Cr (TABLE 1) increased with tannery sludge amendments, but only 10% of total Cr was found to be available, meaning restricted Cr for plant uptake. For Mn, Fe, K, and Zn, there were no significant differences between the control soil and any of the treated soils (P>0.05 Tukey's test). Results for other single extractants than EDTA could be revised in López–Luna et al[10].

Total oxidable trivalent chromium and reactive manganese

Total oxidable Cr^{3+} increased importantly from 6.06 mg/kg in the control soil to 376.24 mg/kg for the maximum tannery sludge amendment (Figure 1), since hypochlorite used in the extraction quickly oxidizes and extracts the inert forms of Cr^{3+} like those from skins tannery process in soil[14], although it should be noticed that along the experiments Cr^{6+} was no longer detected. Cr oxidation rate is determined by speciation and mobility of Cr as well as the aged immobile surfaces of Mn oxides[14]. Accordingly, in this work important reactive Mn levels (124–150 mg/kg) were observed, although without significant differences among treatments (P>0.05 Tukey's test). Interestingly, this total reducible Mn could react with the Cr susceptible to oxidation, increasing Cr mobility and availability for plants and, consequently its accumulation in vegetable tissues. However, if Cr^{3+} is not in a mobile form or that it could be mobilized, it will not be oxidized in spite of optimal manganese oxides surfaces[14]. Because of the alkaline conditions of this tannery sludge amended soil, Cr was expected to easily precipitate as insoluble Cr $(OH)_3$ limiting uptake by plants. Nevertheless, organic complexation of Cr^{3+} has been found to efficiently compete with precipitation, rendering more soluble and therefore more mobile Cr species[17].

Conversely, in metal–contaminated soils, the natural role of metal–tolerant plant growth promoting rhizobacteria in maintaining soil fertility is more important than in conventional agriculture, where greater use of agrochemicals minimize their significance[18]. Besides their role in metal detoxification/removal, rhizobacteria also promote the growth of plants by other mechanisms such as production of growth promoting substances and siderophores[18]. In this work *Rhizobium* nodules were observed in roots of bush bean plants (Figure 2) that

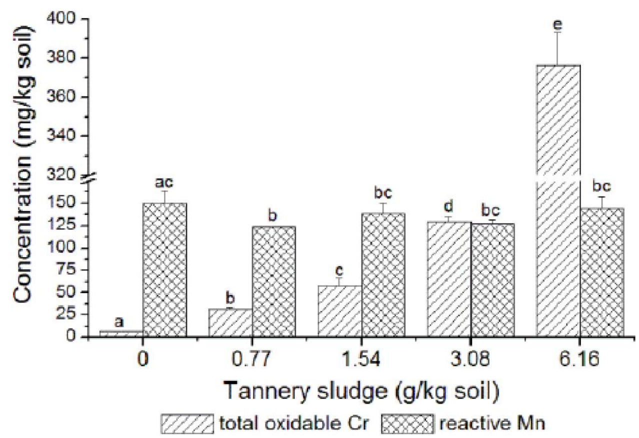

Figure 1 : Total oxidable trivalent Cr and reactive Mn in tannery sludge amended soil. Values are means of three replicates ± standard deviation. Means with the same letter are not significantly different. P<0.05 Tukey's test.

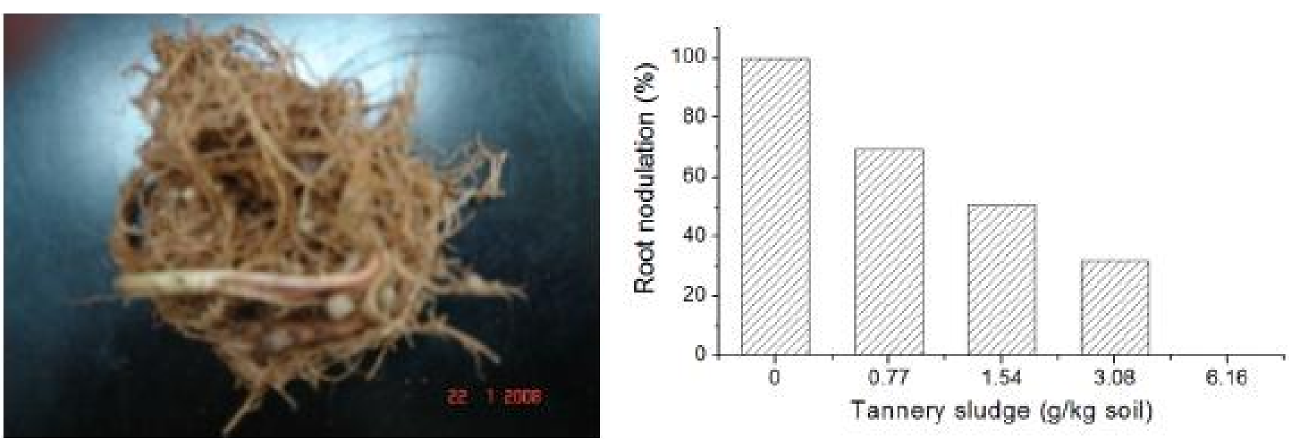

Figure 2 : Root nodulation of bush beans cultivars amended with tannery sludge (expressed as percentage of control soil). The picture corresponds to control plants.

diminished with increasing tannery sludge amendments, as response to Cr toxicity (broadly discussed in López–Luna et al.[10]). Rhizobacterial growth promoting substances and bush bean root exudates could have mobilized Cr through Cr^{3+}-organic complex. Fact explaining the noticeable Cr accumulation in bush bean tissues, despite low Cr availability.

Metal accumulation in vegetable tissues

Metals primarily accumulated in the roots of plants (TABLE 2), with exception of K that tended to be accumulated in the aerial parts of the plants, as it was observed in *Sesamum indicum* (L) grown on tannery–sludge amended soil[19]. Cr accumulation increased with tannery sludge amendments. Significant differences among treatments were not observed for the other metals. Cr accumulation in upper parts of the plants increased with tannery sludge amendments but accumulation only differed significantly in treatments with higher sludge ratios. Mn accumulation was shown not to significantly differ among treatments. Just the opposite was observed for Fe and K, although no clear tendency was observed in the accumulation patterns. Zn and Cd have been shown to be greater incorporated in cultivars grown on sandy soils with low organic matter level[20]. Indeed corroborated in the present work, as Zn primarily accumulated in the roots and was then translocated to the aerial part of the plants, regardless of its low availability. Cr concentration in plant tissues was diminished from the root to the aerial part, being lower in pods and seeds. Cr accumulation in pods increased with tannery sludge amendments, but it was found 50% lesser Cr in seeds. Mn and Fe accumulation was slightly higher in the pods and seeds of control plants than those from tannery sludge amendments. For K, no significant differences were observed in pods, while the greatest accumulation in seeds was observed in plants grown on soil containing 3.08 g of tannery

TABLE 2 : Metal accumulation in bush bean cultivars

Tannery sludge addition (g/kg soil)	Cr (µg/g)	Mn (µg/g)	Fe (µg/g)	K (µg/g)	Zn (µg/g)
Root					
0 control	14.88±1.95 a	235.51±35.58 a	9345.98±1599.90 ab	11168.99±1413.81a	42.53±6.77 a
0.77	260.96±20.70 b	210.64±22.23 ab	8565.96±1469.23 ab	15370.83±155.13 b	40.44±4.01a
1.54	285.86±40.93 b	234.05±17.61 a	10350.79±1994.94 a	11822.18±3259.27 ab	46.76±4.79 ab
3.08	764.26±13.42 c	182.70±13.42 bc	8503.72±1020.62 ab	8404.23±769.58 a	46.50±2.80 ab
6.16	1336.00±7.45 d	154.28±7.45 c	7225.79±861.20 b	8607.79±506.79 a	53.94±6.54 b
Aerial part					
0 control	9.39±3.32 a	51.95±6.39 a	153.72±18.73 a	23909.49±4375.65 ab	28.60±1.03 ab
0.77	9.84±3.38a	47.75±13.24 a	150.65±1.51 a	18912.80±2876.99 b	30.30±4.31 a
1.54	9.62±1.59 a	45.32±8.37 a	103.37±22.68 b	18624.07±1002.19 b	29.20±3.25 a
3.08	28.45±5.75 a	54.50±6.10 a	203.46±2.10 c	25933.93±5953.14 a	28.06±3.19 ab
6.16	1336.00±7.45 d	45.80±9.17 a	207.12±21.12 c	13383.69±5775.11d	23.67±5.02 b
Pods					
0 Control	3.57±1.72a	84.83±14.97a	50.28±9.94a	19407.75±7556.05a	23.41±1.25a
0.77	3.99±1.62a	42.97±10.03bc	32.65±8.60b	28196.00±1844.13a	5.84±0.96b
1.54	6.24±2.07a	37.66±0.33b	37.38±3.60abc	27282.21±465.40a	9.47±0.98b
3.08	8.27±1.75a	54.92±6.90c	29.61±7.84bc	25645.95±6071.62a	5.04±0.41b
Seeds (fruit)					
0 control	1.44±0.98a	35.62±5.53a	30.40±3.60a	11132.27±514.44a	13.01±2.52a
0.77	0.99±0.19a	19.47±5.18b	23.10±4.54a	12175.06±901.48a	14.27±3.94a
1.54	1.17±1.10a	16.67±3.40b	23.30±3.29a	15512.37±4147.82a	12.81±1.56a
3.08	2.58±0.60[a]	28.77±2.48a	30.45±9.18a	19972.81±380.31b	6.69±0.83b

Values are means of four replicates ± standard deviation, in a dry basis. Means with the same letter are not significantly different. *P<0.05* LSD test.

sludge. Zn was mostly accumulated in pods from control plants (23.41 μg/g), diminishing with increasing tannery sludge amendments, but without significant differences among treatments. Finally, for most treatments no significant differences were observed in the accumulation pattern of this metal in seeds.

Relationships between the variables studied

In the correlation matrix (data not shown) from PCA, metal accumulation in pods and seeds was excluded to avoid missing data, since at 6.16 g tannery sludge /kg soil pods and seeds were not formed. Correlation analysis showed that neutral alkaline soil pH and EC favored the increase of total oxidable Cr^{3+} (*r* 0.99, *P*<0.05), thus increasing the EDTA available Cr (*r* 0.97, *P*<0.05) and then Cr accumulation at root level. Conversely, there was no correlation between total oxidable Cr^{3+} and reactive Mn, meaning negligible Cr oxidation despite being Mn oxides the only proven natural oxidants of Cr^{3+} to Cr^{6+} in soils, which increases Cr mobility and toxicity[15]. These results could be supported by the fact that higher pH might reduce reactive Mn oxide surface area, due to precipitation of Cr hydroxide species and enhanced Cr^{3+} sorption caused by increase in total Mn oxides charge, thereby reducing Cr oxidation[21]. Additionally, Fe–Mn reactivity limits Cr oxidation and moreover, Fe oxides are known to effectively reduce Cr. Such conditions might suggest organic complexation as the main Cr^{3+} mobilization mechanism, rendering available Cr for plant uptake despite the hardly noticeable Cr oxidation. A negative correlation was observed between Cr and Mn accumulation in root and upper part of bush beans (*r* -0.79 and -0.84 respectively, *P<0.05*), showing an antagonistic effect. Conversely, Mn and Fe showed a synergistic relationship (*r* 0.85 and 0.74, *P*<0.05).

Principal component analysis (PCA) for grouping variables

PCA of the raw data was conducted using the correlation matrix to identify a reduced number of components that could capture the highest percentage of variance. Four components with eigenvalues =1 were retained (TABLE 3) and eigenvectors with weighty loadings were further on discussed, as these eigenvectors were the most ponderous for PCA. The first principal component (Prin1) explained 55% of the total variance with positive and similar loadings for EC, oxCr, aCr, rCr, and sCr. Similar but negative loadings were recorded for rMn and sMn confirming an antagonistic relationship with Cr. The second component (Prin2) explained 13.85% of total variance with positive loadings for rFe, sFe, and rZn. Observed Zn–Fe synergism is reported to be linked with the P supply. It is suggested that at a relatively high accumulation of P and Zn in roots, the precipitation of $FePO_4$ in root tissues can account for the increase in Fe uptake. Although Zn–Fe antagonistic relationship in plants is more widely known and its mechanism is apparently similar to the depressing effects of other heavy metals on Fe uptake[22]. Negative loadings were observed for rK and sK indicating antagonism with Fe, since K deficiency is also often associated with Fe toxicity[22]. Prin3 and Prin4 were found more related to metal interaction in tannery sludge amended soil and accounted for 10.79 and 8.24% of total variance, respectively. Opposite loadings between reMn and aMn in Prin3 might indicate lesser Mn availability because of Mn reactivity with the other metals and soil minerals. Positive loadings for aK and aZn demonstrated increased availability of these metals in neutral–alkaline soil conditions. Similar but opposed loadings for pH and reMn still sustain the fact that higher pH might reduce reactive Mn oxide surface area, lessening the Mn oxides potential to oxidize Cr, as discussed earlier. The PCA graphic (Figure 3) describes the grouping of variables that exerted main effects on bush beans cultivars amended with tannery sludge. Circled variables (Figure 3a and right side of 3b and 3c) show a common pattern with pH and EC, representing an ideal scenario for Cr oxidation, increasing Cr availability and consequently Cr accumulation in vegetable tissues. However, the last might have been prevented since Cr oxidation by Mn oxides is strongly dependent on pH with the most effective reaction occurring from pH 4.5 to 6.0[23]. Zn accumulation in plant tissues was favored in neutral alkaline soils, as previously mentioned. Fe is known to impair K uptake by plants and reactions between Fe and Mn are commonly observed (left side of Figure 3b and 3c). Indeed, the ratio of these two metals in both growth medium and plant tissues seems to be more important to plant metabolism than their concentrations[22].

TABLE 3 : Principal component analysis (PCA)

Variables	Prin1	Prin2	Prin3	Prin4
pH	0.211	0.006	0.154	0.502
EC	0.300	0.036	-0.098	0.116
oxCr	0.295	0.022	-0.156	0.051
reMn	0.013	-0.004	-0.426	-0.577
aCr	0.300	0.063	-0.071	0.124
aMn	-0.122	0.221	0.397	-0.040
aFe	0.190	-0.145	-0.061	-0.270
aZn	0.164	-0.004	0.532	-0.243
aK	0.164	-0.004	0.532	-0.243
rCr	0.302	0.059	-0.042	0.049
rMn	-0.277	0.174	-0.042	0.020
rFe	-0.195	0.452	-0.022	0.076
rZn	0.160	0.407	-0.019	0.015
rK	-0.212	-0.337	0.004	0.258
sCr	0.302	0.059	-0.042	0.049
sMn	-0.281	0.185	0.025	-0.016
sFe	-0.176	0.471	-0.085	0.083
sZn	0.247	0.185	-0.127	0.200
sK	-0.212	-0.337	-0.127	0.258
Eigenvalue	10.48	2.63	2.05	1.57
Total Variance (%)	55.14	13.85	10.79	8.24
Accumulated (%)	55.14	68.69	79.77	88.01

Prin principal component. Prefixes: ox = oxidable, re = reactive, a = available, r = root, s = aerial part. Eigenvectors with weighty loadings were highlighted.

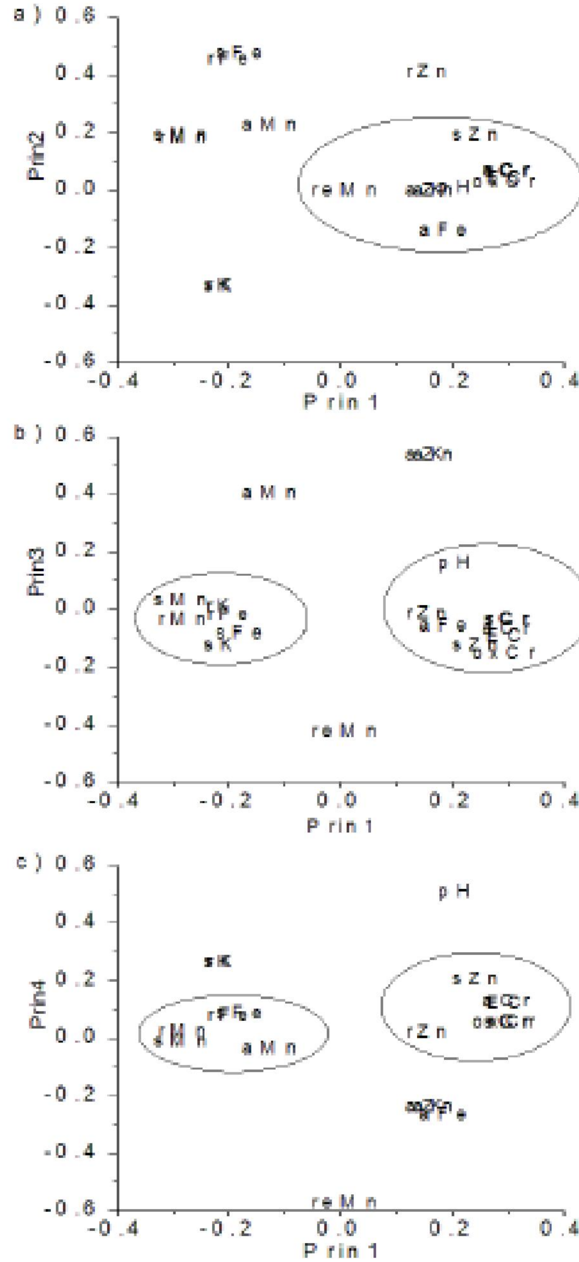

Figure 3 : Principal components ($Prin_{1-4}$) screening the interaction of variables in bush bean cultivars grown on tannery sludge amended soil. Prefixes: ox = oxidable, re = reactive, a = available, r = root, s = aerial part

CONCLUSIONS

Cr availability increased with tannery sludge amendments and Cr^{3+} showed strong tendency to be oxidized. Nevertheless, Cr oxidation by Mn oxides was restricted to more acidic pH range. Metal reactivity was affected by tannery sludge amended soil properties. Most metals were mainly accumulated in roots and then translocated to aerial parts of the plants with minimal Cr accumulation in seeds. Synergistic and antagonistic interactions were observed between metals in soil and plants.

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