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Evaluation of the Phytoavailability of Cu(II) and Cr(III) for the Growing of Corn (*Zea mays* L.), Cultivated in Four Soils of a Toposequence Derived from Basalt

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Abstract

The environmental contamination, from the pollutants generated by industrial development, has been considered, in the last years, one of the most critical environmental problems and worthy of study, mainly regarding the environmental degradation they cause. The use of areas contaminated by heavy metals for agriculture needs information about the behavior of plants and the dynamics of the metal in the soil, to predict the phytodisposability of these metals, present in these areas and their behavior. The present study consists in verifying whether the chemical and mineralogical characteristics of the soils developed in a basalt-derived toposequence can influence the dynamics of Cu^{2+} and Cr^{3+} ions in the phytodisposability of these metals for commercial crops, such as corn. Increasing the dose of the mentioned copper and chromium salts in the NVef and MTF soils, indicates an increase of Cu^{2+} ion in plants. For the Cr^{3+} ion, it accumulated a large amount in the roots, with no translocation occurring to the aerial part. This indicates the potential of Cr^{3+} ion fixation to the organic and inorganic colloids of the soils and its low mobility in the plant. The concentration and accumulation of metals in plants depend on a number of factors, such as soil class, availability of metal in the soil solution, which is related to the weathering process or to anthropogenic contamination, and plant species.

Subject Areas

Agricultural Science

Keywords

Soil Toposequence, Phytoavailability, Heavy Metals, Copper and Chromium

1. Introduction

The use of solid industrial and urban residues as fertilizers and the dumping of these materials on the soil, as well as the excessive use of chemical fertilizers and pesticides have contributed considerably to soil contamination. With this, there are changes in its physical properties and a decrease in the content of organic matter, giving origin to serious consequences as for example, the retention and runoff of water (Drew, 1989) [1].

The toxicity of heavy metals in plants may be the result of complex interactions of metal ions, especially with other soil components or environmental factors. Furthermore, there are few studies on the association between heavy metals and oxidative stress in plants, so it is difficult to make an evaluation about the critical concentration of toxic metal in soils (Gallego, *et al.* 1996) [2].

The concentration of chemical elements in plants depends on the interaction of a number of factors, including soil class, plant species, maturity stage, crop management and climate (McDowell, *et al.* 1993) [3]. However, the main factor is the absorption potential, specific and genetically fixed for the different nutrients and plant species (Mengel, *et al.* 1987) [4]. In addition to these factors, the accumulation of heavy metals is also highly variable from one particular organ of the plant to another (Porto, 1986) [5].

1.1. Relation between Plants and Trace Elements

The concentration and accumulation of metals in plant tissues depend on their availability in the soil solution, and the concentration of metals in the root tissues and in the aerial part increase when their concentration in the soil solution increases. Tolerant species generally accumulate higher concentrations of heavy metals in the root than in the aerial part (Verkleij, *et al.* 1989) [6]. This indicates that plants growing under these conditions cannot avoid uptake of metals, but limit their translocation. Tolerant species can be characterized according to their relative capacity to absorb, translocate and concentrate metals in the plant, being considered accumulators, indicators and excluders, according to the relative concentrations of metals in the roots and in the aerial part (Baker, 1981) [7].

The term heavy metal refers to any chemical element that has metallic properties, atomic number greater than 20 and density greater than 5.0 $g \cdot cm^{-3}$ (Kabata-Pendias, 2010) [8], (Alloway, 2013) [9]. Certain metal with these characteristics can be classified as heavy metal, and does not signify that it is necessarily toxic, and some such as Fe, Mn, Cu and Zn are plant nutrients. Some heavy metals are essential and beneficial, when in adequate concentrations, for the development of plants, when in high concentrations exert toxic effects (Alloway,

2013) [9].

The known or postulated mechanisms to explain the tolerance of plants to heavy metals are grouped into three main categories with several subdivisions (Kabata-Pendias and Pendias, 1984) [10]: 1) limitation in absorption: a) selective exclusion of the metal in the absorption process, b) metal excretion, and c) root excretion of compounds that decrease element availability, 2) compartmentalization: a) metal retention in the root, vessels or both, b) metal immobilization in the cell wall, and c) immobilization of the metal in the vacuole, and 3) biochemical detoxification: a) production by the plant of sequestering and inactivating compounds of toxic metals, and b) tolerance of enzyme systems activated by metals.

Phytotoxic causes of the effects of excessive Cu(II) have been reported in research in recent years. Cu(II), toxic in many non-tolerant plants, may be associated with disturbance in mitosis (Jiang, *et al.* 2000) [11], inhibition of root elongation and damage to root epidermal cell membranes (Ouzounidou, *et al.* 1995) [12].

Cr(VI) has been evaluated to be slightly more toxic than Cr(III) to a bush bean variety (Wallace, *et al.* 1977) [13]. The Cr(III) absorbed by plants is poorly translocated to other parts of the plant (Adriano, 1986) [14]. Pratt (1966) [15], apud Adriano (1986) [14], observed that high concentrations of chromium in plant tissues can cause symptoms of toxicity, starting at approximately 5.0 $mg \cdot kg^{-1}$ for barley, oats and citrus plants and up to 175 $mg \cdot kg^{-1}$ for tobacco.

1.2. Heavy Metal Accumulator Plants

Some plants have the characteristic of accumulating extremely high amounts of some heavy metals without suffering adverse consequences. There are cases in which determined plants serve as indicators of the presence of minerals (Cu, Fe, Mn, Pb, Zn and even Ag and Au). It is not known why such plants act as accumulators. There seem to be genetic factors involved as some related species often have this ability, as shown in **Table 1**.

Plants that have more than 1000 $mg \cdot kg^{-1}$ of heavy metal in their dry matter are called hyperaccumulators, such as *A. bertoloni* and *A. musale* that have 4000

Table 1. Accumulating plants for some heavy metals and other elements.

Element	Content % in ash	Species
Ni	>10	*Alyssumbertolini*
Zn	-	*Thlaspicalaminare*, *Equisetum arvense*
Cr	1 - 3	*Pimeleasuturi*, *Leptospermunscoparium*
Co	-	*Crotalariacobaltica*
Se	-	*Astragalusracimosus*
Sr	-	*Arabisstricta*
U	-	*Uncinialeptostachya*, *Coprosmaaborea*
Cu	0.1 - 1	*Becuimhomblei*
Hg	-	*Betuapapyrifera*
W	-	*Pinus sibiricus*

Source: Kabata-Pendias and Pendias, (1984) [10]; Bereket, (1997) [17]; Okiemen, (1991) [18].

$mg \cdot kg^{-1}$ of Ni in leaves and seeds, and 2500 $mg \cdot kg^{-1}$ in other tissues (Mishra, 1974) [16].

The tolerance to excess heavy metals is highly heritable and persists in seeds produced when tolerant plants are cultivated in uncontaminated soil. The "tolerance" character is generally dominant, depending on the element and species, one or more genes are responsible for tolerance (Gerloff, *et al.* 1983) [19].

The accumulation of metals in plants will depend on factors such as: the species, variety and organ or part studied (Maeda, *et al.* 1990) [20]. Cereals, grasses and legumes tend to accumulate less metal than leafy fast-growing plants such as spinach and lettuce (Lake, 1987) [21].

The plants generally retain most of the heavy metals in their roots. The mobile portion is generally concentrated in the vegetative tissue, and little is translocated to plant reserve organs (Latterel, *et al.* 1978) [22]. Cd(II) and Zn(II), for example, are intensively translocated to the shoot, whereas Cu(II), Cr(III) and Pb(II) are strongly retained in the root, while Ni(II) it is equally distributed by the plant (Matthews, 1984) [23], (Lake, 1987) [21].

The resistance of plants to heavy metals occurs through mechanisms that include immobilization of metal ions in roots and cell wall. The ability to administer, that is, tolerance, in turn, is based on the sequestration of metal ions in vacuoles, bonds with appropriate ligands, such as organic acids, proteins and peptides, and on the presence of enzymes (Garbisu, *et al.* 2001) [24].

There are at least two aspects of practical interest: obtaining genotypes capable of growing and producing in soils with high levels of heavy metals and obtaining genotypes in which the toxic metal is not concentrated in the edible part of the animal, including man. The accumulation of heavy metals up to toxic levels for animals and humans in forage and food is an important aspect to be considered. In this aspect, as described by Ortega (1981) [25], indicating that plants are more resistant to high amounts of heavy metals than animals.

Therefore, to know the contamination, in terms of the effects on plants and the food chain, it is necessary to determine the phytoavailable concentrations of these metals (Leschber, *et al.* 1985) [26]. In **Table 2**, the contents of the metals Cd(II), Cr(III) and Pb(II) are presented.

There are studies in the literature, as described by (Alloway, *et al.* 1993) [27], which prove a strong relation between soil and environmental factors and the availability of metals "trace elements" for plants. **Table 3** shows the concentration considered normal and phytotoxic in soils and plants of heavy metals.

The toxicity of an element must be monitored and verified by the growth rate, productivity, visible symptoms and concentration in the plant tissue (Beckett, 1991) [31]. Some toxicity symptoms caused by heavy metals in plants, are shown in **Table 4**.

1.3. Heavy Metal in Soil

The distribution of toxic elements in the soil profile is variable, due to differences

Table 2. Contents for some heavy metals in foods (Gerloff, *et al.* 1983) [19].

Foods	Cd(II)	Cr(III)	Pb(II)
	---------- mg·kg^{-1} ----------		
Rice	0.05 – 0.34	0.011 - 0.6	0.02
Corn	0.06 - 0.1	0.25	0.02
Wheat (grains)	0.003 - 0.35	0.01 - 0.2	0.037 - 0.16
Wheat (flour)	0.003 - 0.023	-	-
Bean	0.29 - 0.34	0.05	0.02
Lettuce	0.12 - 0.66	0.09 - 0.11	0.7 - 3.6
Tomato	0.03 - 0.23	0.07	1.0 - 3.0
[maximum]*	3 - 8	75 - 100	100 - 400

*Total concentrations of elements considered excessive from a phytotoxic point of view.

Table 3. Concentration of some heavy metals considered normal and phytotoxic in soils and plants in mg·Kg^{-1}.

Metal	CNS[1]	CCTS[2]	CNP[1]	Critical plant concentration	
				A[2]	B[3]
Cd	0.01 - 2.0	3 - 8	0.10 - 2.4	5 - 30	4 - 200
Pb	2.00 - 300	100 - 400	0.20 - 20	30 - 300	-
Cu	2.00 - 250	60 - 125	5.00 - 20	20 - 100	5 - 64
Ni	2.00 - 750	100	0.02 - 5	10 - 100	8 - 220
Zn	1.00 - 900	70 - 400	1.00 - 400	100 - 400	100 - 900

CNS—Normal soil content, CCTS—Critical total soil concentration, CNP—Normal plant content (Bowen, 1979)[1] [28]: A—Toxicity (Kabata-Pendias and Pendias, 1992)[2] [29] and B—Values where the reduction of 10% plant growth (Mcnichol, *et al.* 1985)[3] [30].

Table 4. Main symptoms of heavy metal toxicity in plants.

Metal	Symptoms
Cd	Leaves with brown margins, chlorosis, petioles and reddish veins. Winding of leaves. Brown and short roots.
Co	Iron chlorosis induced in young leaves, whitish tips and margins. Damaged root tips.
Cr	Chlorosis in younger leaves. Poorly developed roots.
Cu	Leaves initially dark green, then chlorosis into watery patches that dry out and may turn almost black. Defoliation. Poorly developed roots. Poor tillering in cereals.
Fe	Dark green leaves. Reduction in shoot and root growth. Leaves can be tan (rice) to reddish.
Mn	Brown or black punctuations on leaves, chlorosis and wrinkling of older leaves. Drying of tips and edges. Poorly developed roots.
Mo	Yellowing or brown color of the leaves. Less tillering of cereals and poorly developed roots.
Ni	Internerval chlorosis of younger leaves or grey-green color. Brown and short roots.
Pb	Dark green leaves, withering of older leaves. Underdeveloped (and brown) aerial parts and roots.
Zn	Chlorosis and tanning of younger leaves. Growth delay. Roots similar to barbed wire.

Source: (Kabata-Pendias and Pendias, 1985) [32].

in the retention capacity of the components, due to the different layers present according to the soil class (Berrow, *et al.* 1980) [33]. As for the origin, the metals present in the soil can be divided into lithogenic and anthropogenic.

The lithogenic fraction comes from geological sources, such as rock residue or is released during the soil weathering process. The natural content of toxic metallic elements in the soil varies greatly with weathering and the chemical composition of the source material. Although the presence of toxic elements, especially heavy metals, be generalized in soils under natural conditions, human activities, that is, anthropogenic action, somehow end up adding to the soil materials containing these elements, which can reach very high concentrations that compromise the quality of the ecosystem. The main anthropogenic sources of metals in the soil are mining, metal processing, the application of pesticides and fertilizers, urban or industrial sewage sludge, reuse water, etc.

Table 5 presents the total and soluble contents of some toxic chemical elements found in the surface layer of soils.

1.3.1. Chrome in Soil and Plants

The chromium metal in the form Cr(III) is the most stable oxidation form of the element in the soil. This form has low solubility and mobility with increasing pH, due to the formation of $Cr(OH)_3$ or even $[Cr(OH)_4]^-$. Although residues from leather tanneries and sewage sludge do not have Cr(VI) in the most oxidized form, its constant accumulation, associated with determined soil conditions, such as the presence of Mn in oxidized forms (Mn^{3+} and Mn^{4+}), is low organic matter contents and good aeration, can promote its oxidation to Cr^{6+} which has high solubility and mobility, with toxic and mutagenic characteristics for higher animals, plants and microorganisms (Milacic, *et al.* 1995) [38].

Plants cultivated in soils with 30 $mmol \cdot Kg^{-1}$ of $CrCl_3$ suffered severe intoxication. The visible toxicity symptoms caused to plants by excessive levels of Cr(III) are: decreased growth, atrophy in radical development, winding and discoloration

Table 5. Minimum and maximum values of the total and soluble contents of some elements in the soil surface layer in $mg \cdot kg^{-1}$.

Element	Lithosphere (1)	Global (2)		São Paulo (3)	
		Total	Soluble	Total	Soluble
Arsênio	-	1 - 50	-	-	-
Pb	16	20 - 500	0.00 - 20	-	-
Co	40	0.05 - 40	0.02 - 5	0.14 - 86	0.01 - 0.58
Cu	70	2 - 100	1 - 8	2 - 340	0.08 - 0.80
Mo	2.3	0.2 - 5	0.0 - 0.2	0.11 - 2.9	0.01 - 0.13
Ni	100	5 - 500	1 - 10	<10 - 126	0.10 -1.40
Zn	80	10 - 300	1 - 20	1 - 315	0.9 - 0.32

Source: (1) Golschimidt, (1958) [34], (2) Pinta, (1977) [35], (3) Furlani, *et al.* (1977) [36] and Rovers, *et al.* (1983) [37].

of the leaves and, in some cultures, leaves with red-brown spots, containing areas of necrosis. The oxidation of Cr(III) to Cr(VI) is favored in soils with pH lower than 5.0. Soils with the presence of easily reducible MnO accelerate this process. These oxides act as electron receptors, functioning as a agent between Cr(III) and oxygen in the atmosphere. As a result of the oxidation process of Cr(III) to Cr(VI), an increase in exchangeable Mn^{+2} can be observed, resulting from the reduction of oxides of this element (Milacic, *et al.* 1995) [38]. Generally speaking, the reaction can be represented by the chemical equation.

$$3MnOOH + Cr(OH)^{+2} + 2H^{+} \leftrightarrow 3Mn^{+2} + [HCrO_4]^{-} + 3H_2O$$

Thus, it appears that the concern about the treatment of effluents rich in Cr(III) is valid. The oxidation reaction takes place and can contaminate not only plants, but all trophic levels, as these are the base of the food chain for all species of life.

As reported by Langlois, *et al.* (2015) [39], the chromium form, that is, the non-toxic Cr(III) form is more often found in soils under normal environmental conditions. The Cr(III) and Cr(VI) species may present oxi-reduction processes, depending on the presence of microorganisms, organic matter, Fe^{3+} or even Mn^{3+}/Mn^{4+} in the forms of oxides or hydroxides.

1.3.2. Copper in Soil and Plants

Cu(II) is an essential nutrient for plant growth when at adequate levels in the soil, it participates in several metabolic processes in plants. As described by (Marschner, 1995) [40], both the deficiency and the toxicity of Cu(II) (Mocquot, *et al.* 1996) [41], cause a reduction in the photosynthetic rate. It is also an important component and activator of several enzymes, performing structural functions in plants, such as opening and closing stomata and plant lignification (Marschner, 1995) [40].

In the soil, Cu(II) is known to have low mobility and, consequently, low availability to plants, especially in soils with fine texture and high content of organic matter. The direct consequence of this behavior is that, even in places with relatively high levels of Cu(II), the low mobility of this element promotes few symptoms of toxicity (Henriques, *et al.* 1993) [42].

The plants absorb Cu(II), which is dissolved in the soil solution, mainly in the ionic form Cu^{2+}, being transported by the xylem in the form of a chelate with amino acids, and its redistribution occurs, depending on its level in the tissue, which does not occur when there is deficiency, as described by (Faquin, 1994) [43]. Even that Cu(II) not involved in redox reactions, its mobility can be drastically affected, due to the increase in pH, concentration of CO_2, S^{2-}, Fe^{2+} and Mn^{2+}, caused by the reduction of the redox potential and all its implications.

The Cu(II) is required in close amounts (5 to 20 $mg \cdot kg^{-1}$) for plant tissue to develop normally (Jones, 1972) [44], while values lower than 4 $mg \cdot kg^{-1}$ are considered deficient and higher than 20 $mg \cdot kg^{-1}$ is considered toxic. The element Cu(II) is a component of several plant enzymes. This occurs as part of the prosthetic groups of enzymes, as an activator of enzyme systems and as a facultative

activator in enzyme systems (Gupta, 1979) [45].

As described by Bussler (1881) [46], reported that the lack of Cu(II) for the plant largely affects physiological processes in plants, such as carbohydrate metabolism (photosynthesis, respiration and carbohydrate distribution), metabolism in the fixation of N_2 and synthesis, protein degradation, cell wall, and especially lignin synthesis.

1.4. Micronutrient Extractors

The availability of chemical elements in soils for plants depends on the process of desorption from the surface of organic and inorganic colloidal particles from the soil to the soil solution, which are physically bound, *i.e.*, electrostatic or chemically bound, which are stronger links.

As described by Hogg, *et al.* (1993) [47], verified that the desorption process depends not only on the total labile metal content of the soil, but also on the soil pH, temperature, element concentration and soil/solution contact time. Metals bound to organic matter are rapidly adsorbed, while desorption is slower. Thus, the release tends to be slow or incomplete due to hysteresis, and the inner sphere complexes require greater activation energy in the desorption process (McBride, 1989) [48].

Regarding the charges derived from complexes, it can be reported that in addition to the structural and proton charges, the charge density of inner sphere complexes can also be defined, which are represented by ionic pairs that have a short-distance bond between the ion and the particle, without interposition of water molecules, that is, it produces specific adsorption effects. The charge density of external sphere complexes, which are represented by ionic pairs having at least one hydration sphere between the ion and the particle, that is, it produces non-specific adsorption effects (Langmuir, 1979) [49].

The complexity of the nature of soil processes and soil-plant relationships is probably one of the biggest reasons for the existence of many methodologies to assess nutrients and heavy metals available to the plant. Most methods are based on establishing a significant correlation between amounts of metals extracted from the soil and contained in plants (Krishnamurti, *et al.* 2000) [50].

The extractors currently used can be grouped into various types according to their properties and mode of action. Chemical extractors are classified as follows: 1) saline $CaCl_2$, KCl, 2) organic EDTA and DTPA; and, 3) acids, such as 0.1 $mol \cdot L^{-1}$ HCl solution, Mehlich-1 and Mehlich-3 acid solution.

The Saline extractors have the ability to extract forms that occur in the soil solution and are weakly adsorbed, while acids extract weakly adsorbed metal contents and part of the chemically adsorbed ones in soil organic and inorganic colloids, and organic ones extract metals that are associated with organic forms and carbonates (Sposito, *et al.* 1982) [51], (Haddad, *et al.* 1993) [52].

Therefore, the objectives of this study are to evaluate the phytoavailability of Cu(II) and Cr(III) in four soils of a basalt-derived toposequence, contaminated

with salts of $CuSO_4 \cdot 5H_2O$ and $Cr_4(SO_4)_5 \cdot (OH)_2$, using corn as an indicator plant, and comparing the efficiency of different metal extraction methods, as follows: 1) distilled and deionized water, 2) Mehlich-1, 3) $CaCl_2$ 0.01 $mol \cdot L^{-1}$ and 4) KCl 1.0 $mol \cdot L^{-1}$, in evaluating the phytoavailability of Cu(II) and Cr(III) for corn plant.

2. Methodology

2.1. Experimental Design

The experiment was carried out in a greenhouse, at the Department of Agronomy, State University of Maringá (UEM), using samples collected from the 0 - 0.2 m layer of four soils of a basalt-derived toposequence in the region of Maringá-PR.

Samples of LATOSSOLO Dystroferric Red (LVdf), NITOSSOLO Red Eutroferric (NVef), CHERNOSSOLO Ferric Clayey (MTf) and VERTISSOLO Hydromorphic Ortic (VGo) were used, as described by (EMBRAPA, 1999) [53]. Since each soil was conditioned in pots with a capacity of 2.5 kg, and treated with salts of $CuSO_4 \cdot 5H_2O$ and $Cr_4(SO_4)_5 \cdot (OH)_2$ at doses of 0.0, 25.0, 50.0, 100.0, 200.0, 400.0 and 800.0 $mg \cdot kg^{-1}$, without drainage and three replicates per treatment.

2.2. Description of the Experiment

Due to its high acidity, the LVdf samples were corrected with $CaCO_3$ PA, increasing the base saturation to the cultivation levels in 70%. The amount of $CaCO_3$ used was 1.16 $g \cdot kg^{-1}$ of soil, being incubated for 10 days in plastic bags at a temperature of 45˚C and another 14 days at room temperature, that is, before the application of Cu(II) and salts Cr(III).

The samples of the four soils, LVdf, NVef, MTf and VGo, after application of copper and chromium salts, $CuSO_4 \cdot 5H_2O$ PA and $Cr_4(SO_4)_5 \cdot (OH)_2$ PA, respectively, were subjected to wetting and drying during 45 days, in order to obtain conditions similar to the field. Afterwards, the samples were ground and sieved in meshes of 2.0 mm of mesh opening and conditioned in the respective vases.

The corn variety planted is a hybrid of Dow AgroScience CO32, where four plants were conditioned per vase, leaving two plants per vase, at the time of thinning, that is, one week after planting.

The amounts of distilled water added, initially, corresponded to 60% of the soil water retention capacity. The irrigation of the vases was subsequently monitored by weighing them, replacing the consumed and evaporated water when necessary. The vases were rotated weekly, in order to minimize the effect of environmental variations to which they were exposed.

Several applications of N, P and K were made to better evaluate the symptoms of deficiency, referring to doses of copper and chromium.

At 14 and 21 days of germination, NPK fertilization diluted in water was applied, in the proportion of 10 cm^3 of the solution per vase, and the salts in the

forms of K_2PO_4 0.04 mol·L^{-1} and NH_4NO_3 0.125 mol·L^{-1}.

At 28 and 35 days after germination, NK fertilization diluted in water was applied, in the proportion of 10 cm^3 of solution per vase, and the salts were in the form of KCl 0.1 mol·L^{-1} and NH_4NO_3 0.125 mol·L^{-1}.

At 35 days after germination, 10 cm^3 of 0.25 mol·L^{-1} K_2SO_4 solution were applied to the vases for sulfur supplementation.

At 42 days after germination, 10 cm^3 of 0.25 mol·L^{-1} $(NH_4)_2SO_4$ solution were applied to the vases for maintenance fertilization.

2.3. Collection Methodology and Plant Analysis

On the 51st day of planting, the aerial part of the corn was harvested (leaves + stalks), being washed and dried in an oven at 65˚C, with air circulation for 72 hours. Subsequently, the material was weighed and ground to evaluate the content of the respective metals Cu(II) and Cr(III), via nitro-perchloric digestion (Sarruge, *et al.* 1974) [54].

2.4. Evaluation of Copper and Chromium in Plant Tissue

Considering that some heavy metals often present in extremely low concentrations, 500 mg of plant material were digested with 10 cm^3 of nitro-perchloric solution in a 6:1 ratio and recovery of the extract in a volume of 50 cm^3 for later analysis.

The roots were also removed from each pot by dismantling the soil and sieved, being washed and dried in an oven at 65˚C, with air circulation for 72 hours, and then ground to evaluate the content of the metals Cu(II) and Cr(III), via nitro-perchloric digestion (Sarruge, *et al.* 1974) [54].

The analytical determination of metals in plant tissue was performed by atomic absorption spectrometry (EAA), model GBC932 AA equipment, using air acetylene flame for Cu(II) and acetylene-nitrous oxide for Cr(III) and wavelength 327.4 nm, 1.0 nm slit for Cu and 528.2 nm, 0.5nm slit for Cr.

The fundamental principle of the atomic absorption spectrometry analysis method involves measuring the absorption of the intensity of electromagnetic radiation, coming from a source of primary radiation, produced by gaseous atoms in the ground state. Currently there are options in the equipment that offers different atomizers that can be flame and graphite furnace.

2.5. Evaluation of Copper and Chromium in Soil

The availability of metals present in the soils was evaluated in function of absorption by maize plants and harvest on the 51st day for soil classes NVef and MTf, intermediate to toposequence, that is, one more weathered and another less weathered.

After corn harvest, the contents of Cu(II) and Cr(III) phytoavailable in the samples (control, 100, 400 and 800 mg·kg^{-1}) were evaluated. The analytical determination of metals in all soil samples was performed by EAA, model GBC932

AA, using air-acetylene flame for Cu and acetylene-nitrous oxide for Cr and wavelength of e 327.4 nm, slit 1.0 nm for Cu and 528.2 nm, 0.5 nm slit for Cr

Avaliação dos Extratores

To evaluate the availability of Cu^{2+} and Cr^{3+} ions in soils, the following extractors were used: 1) distilled and deionized water, 2) Mehlich-1, 3) $CaCl_2$ 0.01 mol·L^{-1} and 4) KCl 1.0 mol. L^{-1}. Duplicate determinations were carried out in samples of NVef and MTf soils, representing a more developed soil class, that is, more weathered and another less developed, respectively.

1) Distilled and Deionized Water

5.0 g of soil were stirred with 50 cm^3 of distilled and deionized water in a 250 cm^3 Erlenmeyer flask for 1 hour at 160 rpm and rest for an equal period. Afterwards, the supernatant was centrifuged at 2000 rpm, for 10 minutes, collecting a 25 cm^3 aliquot, kept in a dark flask, for later evaluation of Cu^{2+} and Cr^{3+} ions.

2) Mehlich-1

5.0 g of soil were stirred with 50 cm^3 of Mehlich-1 solution in a 250 cm^3 Erlenmeyer flask for 1 hour at 160 rpm and rest for the same period. Afterwards, the supernatant was centrifuged at 2000 rpm for 10 minutes, collecting a 25 cm^3 aliquot, kept in a dark flask, for later evaluation of Cu^{2+} and Cr^{3+} ions.

3) $CaCl_2$ 0.01 mol·L^{-1}

5.0 g of soil were stirred with 50 cm^3 of $CaCl_2$ 0.01 mol·L^{-1} solution in a 250 cm^3 Erlenmeyer flask for 1 hour at 160 rpm and rest for the same period. Afterwards, the supernatant was centrifuged at 2000 rpm for 10 minutes, collecting a 25 cm^3 aliquot, kept in a dark flask, for later evaluation of Cu^{2+} and Cr^{3+} ions.

4) KCl 1.0 mol·L^{-1}

5.0 g of soil were stirred with 50 cm^3 of KCl 1.0 mol·L^{-1} solution in a 250 cm^3 Erlenmeyer flask for 5 minutes at 160 rpm and rest for 12 hours. Afterwards, the supernatant was centrifuged at 2000 rpm for 10 minutes, collecting a 25 cm^3 aliquot, kept in a dark flask, for later evaluation of Cu^{2+} and Cr^{3+} ions.

5) Sulfuric Digestion

The medium-sulfuric digestion was carried out in air-dried fine earth, in duplicates in the NVef and MTf classes, respectively. After corn harvest on the 51st day of planting in the samples (control, 100, 400 and 800 mg·kg^{-1}) respectively.

0.5 g of soil was added in 100 cm^3 test tubes, together with 20 cm^3 of 1:1 H_2SO_4 solution. The material boils for 30 minutes. Afterwards, 1 cm^3 of 65% HNO_3 was added, until complete digestion of the organic matter. Afterwards, the samples were heated for another hour at a temperature of 250˚C. Then the solution was transferred to a stock flask, increasing the final volume to 50 cm^3.

The Cu^{2+} and Cr^{3+} ions were determined by EAA, as described by (EMBRAPA, 1997) [55].

2.6. Determination of Suspension pH

The determination of pH was carried out directly in the soil-solution suspension, after carrying out each procedure using the different extractors listed

above, with the solutions of Cu^{2+} and Cr^{3+} ions. According to methodology (EMBRAPA, 1997) [55].

3. Results and Discussion

The soils used in the experiment show high variability in some characteristics that can affect the availability of Cu(II) and Cr(III) ions, such as pH, clay content (%), total specific surface area (ASSt), capacity for cation exchange (ECC) and mineral fraction (type 2:1), such as smectites, are shown in **Table 6**.

It can be observed that the more weathered soil classes, that is, LVdf and NVef, although have higher clay contents (70.64% and 69.17%, respectively), do not indicate the presence of 2:1 minerals (smectites), sufficient for high adsorption capacity, as observed for the other classes present in the toposequence, indicating that these initial classes of the toposequence have a high stage of development, that is, a well-defined oxidation state. It can be verified by the presence of kaolinite and gibbsite in the clay composition, as described by (Peternele, *et al.* 2014) [56].

3.1. Dry Mass Production

The toposequence soils with the chemical element Cu(II) present at defined concentrations in the experiment, were favorable for dry matter production up to levels of 25 $mg \cdot kg^{-1}$ for MTf class and 50 $mg \cdot kg^{-1}$ for VGo. Classes LVdf and NVef, showed loss of production from the first dose, indicating that possibly the soils have high levels of pre-existing Cu(II), whose added values become phytotoxic.

The VGo class was the one that produced more shoot dry matter of corn, in treatments with doses of Cu(II) and Cr(III), due to its own fertility characteristics.

Cr(III) was less phytotoxic at the same doses used for Cu(II), and a corn plant tolerance at levels of approximately 400 $mg \cdot kg^{-1}$, except for the NVef class, observed at 200 $mg \cdot kg^{-1}$, indicating metal retention in the roots of maize plants, which is in agreement with Matthews (1984) [23] and Lake (1987) [21].

Table 6. Physical and chemical characteristics (0 - 0.2 m) of the soils LATOSSOLO Dystroferric Red (LVdf), NITOSSOLO Eutroferric Red (NVef), CHERNOSOIL Ferric Clayey (MTf) and VERTISSOLO Hydromorphic Ortic (VGo), such as pH, total specific surface area (ASSt), clay content (%), capacity for cation exchange (ECC) and mineral fraction (type 2:1).

Soil	*pH*		ASSt	Clay	ECC	Mineral
	H_2O	- $CaCl_2$	$m^2 \cdot g^{-1}$	%	$cmolc \cdot dm^3$	2:1
LVdf	5.50	4.70	71.79	70.64	12.29	3.53
NVef	6.30	5.50	106.74	69.17	21.77	8.29
MTf	6.20	5.50	131.82	40.97	43.28	16.33
VGo	6.00	5.10	246.81	58.44	64.08	36.11

Source: (Peternele, *et al.* 2014) [56].

Doses above 400 mg·kg^{-1} of Cu(II) and Cr(III) strongly affect the development, and consequently the production of dry mass of corn, indicating that the levels of toxicity were reached to the point of plant damage. Except for Cr(III) in LVdf and VGo classes with low reduction in dry mass production for the dose of 800 mg·kg^{-1}.

Table 7 shows the amount of corn dry matter produced in different soils and doses of Cu(II) and Cr(III) metals.

It can also be seen in Table 7, the effect of different doses of Cu(II) was highly harmful to the development of the maize plant, in the four soils of the basalt-derived toposequence compared to Cr(III), where the experiments with the chemical element Cr(III), for the same soils showed to be more tolerant.

3.2. Plant Tissue Evaluation

It can be seen in Table 8 and Table 9, that both Cu(II) and Cr(III) were absorbed by the corn plant, as a function of increasing doses of the respective metals, verifying that Cu(II) was more absorbed and accumulated in the root system and translocated in smaller amounts to other parts of the plant, the same not happening with Cr(III), where there was a smaller absorption by the root system that was retained in it, more specifically in the vacuole, as described by (Garbisu, *et al.* 2001) [24].

The greater absorption of Cu(II) by the maize plant verified for the LVdf class, which also produced the smallest amount of dry matter, can be explained by the effect of the liming that this soil underwent before planting, favoring greater absorption.

It can also be observed that the average contents of Cu(II) found in the aerial part of the corn plant for the LVdf, NVef and MTf classes are within the range considered adequate of 6 - 20 mg·kg^{-1}, as per described by (Raij, *et al.* 1996) [57], exception to these values were observed in the VGo class in the control treatments, that is, 0.0 dose of Cu(II), whose concentration was from (3.56 mg·kg^{-1} to

Table 7. Corn plant aerial part dry matter after the 51st day of planting for soil classes, LVdf, NVef, MTf and VGo as a function of Cu(II) and Cr(III) dose.

Dose	LVdf	NVef	MTf	VGo	LVdf	NVef	MTf	VGo
mg·kg^{-1}	dry matter (g) – copper vase*				dry matter (g) – chrome vase**			
0	5.30	8.14	8.50	9.69	5.30	8.14	8.50	9.69
25	4.75	6.74	10.06	9.60	5.41	8.24	8.23	11.85
50	4.74	5.68	8.33	11.17	5.51	7.58	8.31	11.94
100	3.78	5.20	7.49	9.98	5.01	7.57	10.26	12.55
200	3.00	3.51	5.93	7.84	4.38	7.23	10.36	11.77
400	1.96	2.04	3.76	3.97	4.73	5.94	11.92	11.00
800	0.16	1.27	1.33	0.83	4.38	0.99	3.51	8.62

Values represent the average of 3 replicates. (*) Copper outline and (**) Chrome outline.

Table 8. Mean Cu(II) concentration in aerial part and corn roots for soil classes LVdf, NVef, MTf and VGo as a function of metal dose.

Part of the Plant	Dose ($mg \cdot kg^{-1}$)						
	0	25	50	100	200	400	800
				LVdf			
Aerial part	9.46	11.33	12.00	16.53	24.20	44.00	124.53
Roots	87.50	108.29	173.83	243.56	330.22	355.35	591.21
				NVef			
Aerial part	5.03	6.33	8.13	10.76	15.13	23.70	32.23
Roots	88.71	152.89	186.70	225.32	274.97	432.42	520.24
				MTf			
Aerial part	7.03	7.16	7.26	11.73	18.43	27.33	29.96
Roots	70.90	117.29	154.42	214.61	249.32	386.84	555.11
				VGo			
Aerial part	3.56	4.33	4.43	4.56	7.00	14.16	27.33
Roots	28.22	29.62	29.96	59.16	181.34	343.54	517.86

Aerial part (leaf + culm): represents the average of 3 repetitions. Roots part: represents the average of 2 repetitions.

Table 9. Mean Cr(III) concentration in aerial part and corn roots for soil classes. LVdf. NVef. MTf and VGo, as a function of metal dose.

Part of the Plant	Dose ($mg \cdot kg^{-1}$)						
	0	25	50	100	200	400	800
				LVdf			
Aerial part	ND	ND	ND	ND	ND	ND	ND
Roots	7.42	10.87	17.13	22.68	30.45	56.64	129.86
				NVef			
Aerial part	ND	ND	ND	ND	ND	ND	ND
Roots	2.45	6.78	11.15	17.78	63.45	121.03	285.17
				MTf			
Aerial part	ND	ND	ND	ND	ND	ND	ND
Roots	1.28	19.19	37.70	57.21	68.63	199.05	414.23
				VGo			
Aerial part	ND	ND	ND	ND	ND	ND	ND
Roots	ND	ND	ND	2.45	5.06	33.46	83.19

Aerial part: represents the average of 3 repetitions; Root part: represents the average of 2 repetitions; ND = not detectable in the sensitivity limit of the EAA.

4.56 $mg \cdot kg^{-1}$) up to the dose of 100 $mg \cdot kg^{-1}$ of the metal and increasing for the other doses. In general, increasing values of Cu(II) content are observed for all classes of soils in the toposequence as a function of the applied dose, both for the

aerial part and for the root system in the corn crop. This can be explained by the difference in the mineralogy of each class and its physicochemical attributes between the classes, where the metal is available in a different way for plants, considering that the organic matter content for the studied soil classes it is between 14.08 and 16.69 $g \cdot dm^{-3}$, that is, values relatively close, as described by Peternele, *et al.* (2014) [56].

Table 9 shows the absence of Cr(III) in the aerial part of corn for all soil classes and rates, which was also verified by Anjos and Mattiazzo (2001) [58].

It can also be observed that Cr(III) presented low mobility in the soil classes of the toposequence, indicating low absorption by the maize plant and still located in the roots, as a natural defense mechanism by the plant, which was also verified by Garbisu, *et al.* (2001) [24].

In Table 9, it can also be observed that the soil class that made less Cr(III) available in solution was VGo, indicating that the chemical, physical and mineralogical characteristics of each soil have a strong influence on the retention capacity of these metals.

3.3. Extractor Evaluation

The evaluation of the phytoavailability of metals to maize plants was evaluated for Cu(II) and Cr(III), for intermediate soil classes of the basalt-derived toposequence, that is, a more developed class (NVef) and a less developed (MTf).

The amounts of Cu(II) and Cr(III), obtained by the extractors of the samples treated with increasing doses of these metals, after harvesting on the 51st day of planting, are shown in Table 10. Considering the amount of metals added to the soil, in form of salts, it is possible to estimate that the average amount of Cu(II) and Cr(III) extracted by the different extractors in the respective treatments, knowing that the control, that is, the soil without addition of metal presents an initial concentration of the respective metals Cu(II) and Cr(III).

It is observed in Table 10 that the total content of metal ions present in the two soil classes can be determined from the sulfur attack experiment, that is, the values obtained in each dose of the experiment compared to the control. It can also be observed that the extractors used in the extraction of the respective Cu(II) and Cr(III) ions for the two soil classes evaluated presented results that do not correspond to a recovery of added metals in the form of salts, that is, considering the total original content of the soil plus that added via salinization in that treatment at different doses. Indicating that part of the metal went to the corn plant and most of it was chemically and physically fixed in clay minerals, whose mineralogy is characteristic of each class. Also observed by Warman, *et al.* (2000) [59].

Physical and chemical processes cause metals to be in soluble form, fixed by minerals in the soil, precipitated with other components, in biomass and complexed with some components of organic matter. Thus, a given metal present in the soil solution has its balance related to clay particles, iron oxyhydroxide,

Table 10. Contents of Cu(II) and Cr(III), at different doses extracted by sulfuric attack, Mehlich-1, solutions of $CaCl_2$ 0.01 mol·L^{-1}, KCl 1 mol·L^{-1} and distilled and deionized water, observed within each treatment for the NVef and MTf soil classes.

Methods	Dose (mg·kg^{-1})	NVef	MTf	NVef	MTf
		Cu (II) in mg·kg^{-1}		Cr (III) in mg·kg^{-1}	
Sulfuric attack	0	350.35	311.00	35.70	36.80
	100	406.20	355.45	97.00	93.90
	400	794.20	509.35	318.05	304.15
	800	1087.25	864.65	551.40	518.95
Mehlich-1	0	43.78	26.64	0.74	0.00
	100	101.475	70.24	2.53	3.07
	400	308.17	293.89	9.42	8.52
	800	478.17	475.44	32.44	16.00
KCl 1 mol·L^{-1}	0	1.91	3.45	ND	ND
	100	3.03	4.22	ND	ND
	400	15.24	11.58	ND	ND
	800	43.05	34.85	ND	ND
Distilled and deionized water	0	0.42	0.28	ND	ND
	100	0.45	0.90	ND	ND
	400	0.98	1.23	ND	ND
	800	2.76	3.06	ND	ND
$CaCl_2$ 0.01 mol·L^{-1}	0	0.38	0.41	ND	ND
	100	0.68	0.53	ND	ND
	400	4.30	3.44	ND	ND
	800	14.72	11.96	ND	ND

Values represent the average of 2 repetitions. ND—not detected.

aluminum and manganese, in addition to soluble chelators, as described by (Warman, *et al.* 2000) [59].

The greater amount of metals extracted in NVef and MTf soils is mainly due to the richness of metals in the source material of these soils using the extractor from digestion with sulfuric acid.

Comparing the two soils of the NVef and MTf experiment, with all extractors, except sulfur attack, it is verified that the extraction with Mehlich-1 removed higher contents of Cu(II) and Cr(III) in treatments at different doses.

Cr(III) was not detected in the extracts from the treatments of the samples of two evaluated soil classes, that is, NVef and MTf using the extractors KCl 1 mol·L^{-1} solution, deionized distilled water and $CaCl_2$ solution 0.01 mol·L^{-1}, being detected at low levels when extracted with Mehlich-1, which highlights the importance of soil type in evaluating the efficiency of each extractor.

The pH values of the suspensions for the different extractors are also shown in Table 11. Knowing that most extractors acidify the soil, reproducing the behavior

Table 11. Suspension pH values for different extractors at room temperature for the toposequence soil classes derived from Basalt NVef and MTf.

Methods	Dose ($mg \cdot kg^{-1}$)	NVef	MTf	NVef	MTf
		Cu(II) $mg \cdot kg^{-1}$		Cr(III) $mg \cdot kg^{-1}$	
Mehlich-1 (pH = 1.42)	0	1.53	1.53	1.53	1.53
	100	1.53	1.53	1.51	1.53
	400	1.53	1.53	1.51	1.53
	800	1.53	1.53	1.51	1.53
Solution KCl 1 $mol \cdot L^{-1}$ (pH = 5.96)	0	5.08	4.97	5.08	4.95
	100	5.11	4.95	4.87	4.79
	400	4.96	4.85	4.77	4.68
	800	4.98	4.88	4.65	4.68
Distilled and deionized water (pH = 5.25)	0	6.08	6.00	6.08	6.00
	100	5.97	5.83	5.75	5.72
	400	5.61	5.54	5.60	5.44
	800	5.57	5.43	5.35	5.34
Solution $CaCl_2$ 0.01 $mol \cdot L^{-1}$ (pH = 5.70)	0	5.30	5.20	5.30	5.20
	100	5.25	5.20	5.12	5.02
	400	5.17	5.05	5.04	4.95
	800	5.17	5.05	4.97	4.95

Values represent the average of 2 repetitions.

of plants in order to absorb the nutrient. It can be observed that there is practically no variation in the pH result of the extractions using the Mehlich-1 extractor.

Table 11 also shows that the difference in pH between the soils also occurred in relation to the metals Cu(II) and Cr(III) for the different extractors, except for the Mehlich-1 extractor. Clearly indicating that the reaction of the soil with the extractor is an important factor in the availability of metals. Thus, it seems convenient that the extractor has the ability to discriminate the effect of pH on these metals availability in different soils.

Most Brazilian soils are acidic, in this condition, the results are not very conclusive in predicting phytoavailability, also observed by Anjos & Mattiazzo, (2001) [58]. The low correlations are due to the fact that the extractors used do not simulate the reactions that occur in the rhizosphere, as descibed by Berton, (2000) [60].

4. Conclusion

The present study mainly investigated the phytoavailability of Cu (II) and Cr (III) for the corn plant. The increase in the dose of copper and chromium salts added to the four soils of the toposequence derived from basalt, indicates an in-

crease in the Cu^{2+} ion throughout the plant, differently the absorbed Cr^{3+} ion was located in the vacuole of the maize plant. The results show that the concentration and accumulation of metals in plants depend on several factors.

The addition of Cu(II) and Cr(III) in doses applied to soils showed a tendency to a reduction in the production of corn biomass.

The maize plant concentrated greater amounts of Cu(II) than Cr(III) in the roots as a function of the dosage of the respective metals, thus showing the potential for fixation of Cr(III), also to organic and inorganic colloids in the studied soils and its mobility only in the root system of the maize plant.

Among the evaluated extractors, the Mehlich-1 extractor was the most efficient to verify the phytoavailability of Cu(II) and Cr(III) compared to others evaluated under the same conditions. The low efficiency of these extractors is due to their own chemical characteristics, considering the acid tropical soils, the extractors do not simulate the reactions that occur in the rhizosphere.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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