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Nickel Effects on Growth and Phytolith Yield of Grasses in Contaminated Soils

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Abstract: Nickel (Ni) is extremely toxic to plants at high concentrations. Phytoliths have the potential to sequester the heavy metals absorbed by plants and act as a detoxification mechanism for the plant. The authors of the present study aimed to evaluate the effects of Ni on the growth and phytolith yield of grasses in two artificially contaminated soils. Two experiments separated by soil types (Typic Quartzipsamment and Rhodic Hapludox) were conducted in a completely randomized design in a 2 × 4 factorial scheme with three replications. The factors were two species of grass (*Urochloa decumbens* and *Megathyrsus maximus*) and three concentrations of Ni (20, 40, and 120 mg kg^{-1}) and control treatment. The grasses were influenced by the increase in Ni rates in the soils. Ni exerted a micronutrient function with the addition of 30 mg kg^{-1} of Ni in soils, but this concentration caused toxicity in grasses. Such a level is lower than the limits imposed by the Brazilian environmental legislation. Higher Ni availability in Typic Quartzipsamment promoted Ni toxicity, with reduced growth and increased phytolith yield in the shoot, increased Ni in the shoot, and Ni occlusion in phytoliths by grasses, in comparison with Rhodic Hapludox. The yield and Ni capture in phytoliths by grasses in Ni-contaminated soils are related to the genetic and physiological differences between grasses and Ni availability in soils. Ni capture by phytoliths indicates that it may be one of the detoxification mechanisms of *Urochloa decumbens* to Ni contamination, providing additional tolerance. *Megathyrsus maximus* may be a future grass for the phytoremediation technique in Ni-contaminated soils.

Keywords: heavy metal; *Urochloa*; *Megathyrsus maximus*; entisol; oxisol; phytolith

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1. Introduction

Nickel (Ni) is a micronutrient for normal plant growth and development [1,2] and is required in very small concentrations for optimum plant growth [2]. It is extremely toxic in high concentrations and is among the most common heavy metals in soils [1,2].

The phytoremediation technique uses the potential of some plants to tolerate heavy metals for the restoration of contaminated soils [3]. Therefore, tolerant species can be characterized according to their ability to uptake, translocate, and concentrate metals in the plant [3,4]. Depending on the criteria, a plant may or may not be considered a phytoextractor of metal [3,4]. In general, critical toxicity levels of Ni are 10 mg kg^{-1} dry matter in sensitive species, 50 mg kg^{-1} dry matter in moderately tolerant species, and 100 mg kg^{-1} dry matter in tolerant species [5].

Phytoliths represent biomineralizations comprising either silica or calcium, which undergo precipitation within cellular structures or intercellular spaces of plant tissues [6–9]. The deposition of phytoliths confers upon plants the capacity to ensnare and neutralize deleterious metal ions, which is particularly evident in certain plant species belonging to the Cyperaceae and Poaceae families [6,9]. Certain members of the Poaceae family demonstrate resilience to an accumulation of toxic metals [3,4], characterized by elevated rates of growth and substantial biomass yield capabilities, coupled with heightened phytolith production [6–9].

The grasses scrutinized in this investigation encompass species such as *Megathyrsus maximus* and *Urochloa decumbens*, exhibiting noteworthy biomass generation and swift growth rates [1]. Grasses are categorized based on their handling and soil fertility prerequisites [10]. *Megathyrsus maximus* has attained global recognition for its elevated productivity and adeptness in adapting to various soil and climatic contexts [10], whereas *Urochloa decumbens* grasses distinguish themselves for their ruggedness and commendable adaptability across diverse environments [11]. However, the phytolith production capacity of these grasses remains undetermined.

The Brazilian National Environmental Council recently established investigation values for metals in agricultural soils, including Ni [12], without specifically mentioned soil type. However, soil type plays a key role in controlling the heavy-metal availability for plant uptake [7,13,14]. Phytolith yield in plants is not yet fully understood; however, yield is related to climatic conditions, plant age and taxonomy, evapotranspiration rate, soil type, and heavy-metal availability [7,15]. Therefore, the relationship between Ni availability and phytolith yield in grass to select grasses with phytoremediation potential in Ni-contaminated soils has not been studied yet. Given the above, we aimed to evaluate the effects of Ni on the growth and phytolith yield of grasses in two artificially contaminated soils.

2. Material and Methods

2.1. Soil and Experimental Design

Two greenhouse experiments were carried out in Diamantina (18°15′ S, 43°36′ W, 1250 m a.s.l.), located in the state of Minas Gerais, Brazil. The soils under investigation were identified as Typic Quartzipsamment (TQ) and Rhodic Hapludox (RH) following Soil Taxonomy [16], displaying distinct chemical and textural attributes. These soils were sourced from their native "Cerrado" (savanna) state to guarantee the absence of metal contamination in the superficial soil layer (0–0.2 m depth). A subsample was procured, subjected to air-drying, and sieved at 2.0 mm for subsequent chemical analysis and determination of soil texture [17] (Table 1). The total Ni concentration was evaluated by the USEPA 3052 method using microwaves with a digestion solution of H_2O_2 + HNO_3 + HF and H_3BO_3 [18] (Table 1).

Liming was addressed using dolomitic limestone with 38.0% CaO, 12.5% MgO, and 90% total neutralizing power. The liming calculation was executed employing the base saturation method, utilizing the subsequent formula: Liming requirements (Mg ha^{-1}) = ((Vg − Va) × CEC)/100, where Vg signifies the recommended base saturation for grasses (45%), Va denotes the base saturation determined in soil analysis, and CEC represents the cation exchange capacity (Table 1). Application of limestone to the soils occurred, allowing for a 30-day reaction period under soil moisture conditions at field capacity. Monitoring of soil moisture levels was conducted through daily weighing of the pots.

Table 1. Chemical attributes and soil texture before applying treatments.

Attribute	Unit	Soil	
		Typic Quartzipsamment (TQ)	**Rhodic Hapludox (RH)**
pH [(a)] $_{water}$	-	5.1	5.2
P [(b)]	mg kg^{-1}	0.2	0.2
K [(b)]	$mmol_c$ kg^{-1}	0.4	0.2
Ca [(c)]	$mmol_c$ kg^{-1}	6.7	4.5
Mg [(c)]	$mmol_c$ kg^{-1}	3.5	1.8
Al [(c)]	$mmol_c$ kg^{-1}	7.8	4.2
Cation-exchange capacity (CEC)	$mmol_c$ kg^{-1}	40.6	71.5
Base saturation	%	26.0	9.0
Organic carbon [(d)]	g kg^{-1}	3.5	5.8
Ni [(e)]	mg kg^{-1}	<0.1	<0.1
Maximum P adsorption capacity	mg kg^{-1}	100	250
Fe_2O_3 [(f)]	g kg^{-1}	7	298
Al_2O_3 [(f)]	g kg^{-1}	33	166
Clay [(g)]	g kg^{-1}	60	510

[(a)] Soil/water 1:2.5. [(b)] Mehlich-1 extractor. [(c)] KCl 1 mol L^{-1} extractor. [(d)] Walkley–Black method. [(e)] USEPA 3052 method. [(f)] Sulfuric acid digestion. [(g)] Pipette method.

The basal fertilization rates adhered to recommended guidelines [1], except phosphate fertilization, where the fertilization rate was determined based on the maximum phosphorus adsorption capacity of each soil (Table 1), extrapolated from Langmuir isotherm second region data [19]. Consequently, the applied phosphorus rates were 200 mg for Typic Quartzipsamment (TQ) and 450 mg for Rhodic Hapludox (RH) per kg of soil, utilizing NaH_2PO_4 as the phosphorus source. Nutrients were administered in the form of pure analytical-grade reagents, thoroughly mixed, and allowed to incubate for 15 days in each soil. Nickel concentrations, post-liming and basic fertilization, were introduced as nickel chloride with analytical-grade chemical reagent standards. The concentrations were meticulously incorporated into the soil within a 5 kg plastic bag and subsequently transferred to pots for a 15-day reaction in the soil.

Two distinct experiments, segregated by soil type (TQ and RH), were established employing a completely randomized design, distributed in a 3 × 4 factorial arrangement with three replications in polyvinyl chloride pots weighing 4 kg and filled with 3 kg of soil. The experimental treatments comprised the combination of two grass species (Urochloa decumbens (Stapf) R.D. Webster cv. Basilisk and Megathyrsus maximus (Jacq.) B.K. Simon and S.W.L. Jacobs cv. Mombaça) and three nickel (Ni) concentrations (20, 40, and 120 mg kg^{-1} of soil), along with a control treatment involving no addition of Ni. These concentrations correspond to 0.3, 0.6, and 1.8 times the investigated values in the soils [12].

Soil samples were taken from each pot one day before sowing. Sampling was carried out with a polyvinyl chloride pipe of a half inch 0.40 m in length, with four holes throughout the length of the pot with soil. Subsequently, the soil samples were air-dried and sieved through sieves of <2 mm in diameter. The available Ni concentration in soil was extracted with Mehlich-1 [14].

Five seeds from each grass species were sown in pots. Seedlings were thinned to one plant per pot within 14 days after emergence and the four top-dressing fertilizations of 50 mg N (urea) per kg soil every 15 days, after the thinning of grasses. The pots were irrigated daily with distilled water to maintain soil moisture at field capacity with daily checks by weighing the pots throughout the experimental period.

2.2. Measurements and Analytical Determinations

After 120 days of thinning of grasses, the plants were harvested from the shoot. Shoot samples were washed three times in deionized water and dried in a forced ventilation oven at 65 °C until constant weight, with subsequent weighing to obtain shoot dry weight. A microwave oven (CEM MarsTM 6) with nitric acid (65% *v*/*v*—Merck, Rahway, NJ, USA) was used to extract Ni from the shoots of the grasses. Graphite furnace atomic absorption spectrometry (Perkin–Elmer Analyst 800) was used to determine the Ni concentration in the aerial part of grasses. Quality control of Ni analysis used certified reference material (NIST SRM 1573a Tomato leaves) with a recovery rate of 98 $\pm$ 2.

The extraction of phytoliths from the shoot of grasses as prepared and separated was performed using the process adapted from [20]. The concentration of phytoliths was determined by weighing 10 g of dry mass from the crushed aerial part in a porcelain crucible which was subsequently subjected to calcination at 600 °C in a muffle furnace for six hours. The resulting ashes were transferred to Falcon tubes, where carbonates were removed by the application of 2.5 mL of 1 mol L^{-1} HCl. The ashes were then purified using 2.5 mL of hydrogen peroxide (H_2O_2) of 30 volume. The residue underwent consecutive washes with distilled water, followed by centrifugation at 300 rad s^{-1} for five minutes, with the supernatant being discarded. This procedure was repeated in five cycles. The resulting residue (silica phytolith) was dried at 105 °C in a drying oven until a constant weight was achieved. Quantification was conducted through classical gravimetry, employing a precision analytical balance with an accuracy of 0.00001 g. The phytolith concentration results were expressed in g kg^{-1} of the initially crushed dry mass.

Nickel was extracted from phytoliths using the USEPA 3052 method, with digestion in a microwave oven (CEM MarsTM 6) with H_2O_2 + HNO_3 + HF and the addition of H_3BO_3 [18]. Atomic Absorption Spectrometry using a graphite furnace (AAnalyst 800, Perkin-Elmer, Waltham, MA, USA) was used to determine the Ni concentration in the filtered solutions of phytoliths extracted from the shoots of grasses.

2.3. Statistics

The normality and homoscedasticity of the variables evaluated were assessed before the analysis of variance. The data were subjected to an analysis of variance, which consisted of the study of Ni concentrations and the control and the grasses in each soil type. This means that the grasses were compared by Tukey's test at 5%. The regression equations were adjusted for the variables depending on the Ni concentrations added to the soil. Statistical analyses were performed using Sisvar v. 5.6 [21] and the graphs were plotted in SigmaPlot v. 10.0 (Systat Software Inc., Chicago, IL, USA).

3. Results

3.1. Soil Characteristics

The soils exhibited highly contrasting chemical and textural characteristics (Table 1). The TQ soil exhibited much lower clay, Fe and Al concentrations, yielding a lower CEC than the RH soil. Both soils exhibited no Ni contamination in natural conditions. Despite the different chemical attributes between the two soils, the available concentration of Ni increased linearly with the applied concentrations in both soils (Figure 1). The Ni availability, based on the regression coefficients of the adjusted equations (Figure 1), indicates that in each kg of soil available, there was 0.68 mg of Ni in the TQ soil and 0.48 mg in the RH soil, with the Ni availability in the TQ being 42% higher than in the RH soil.

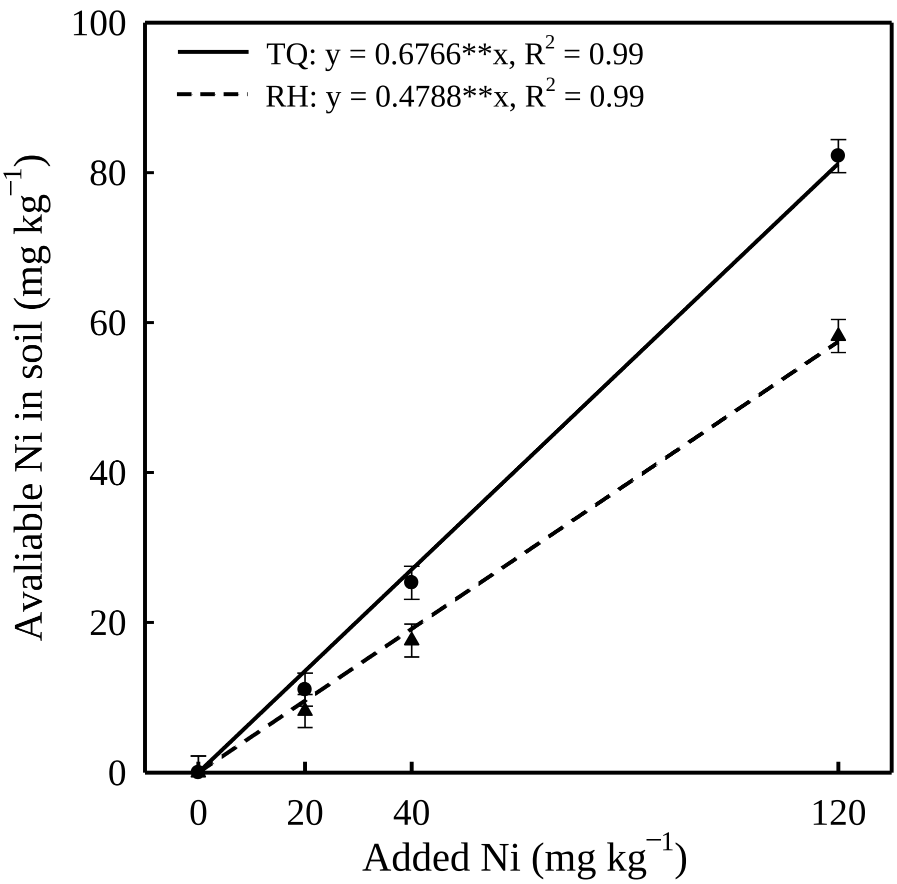

Figure 1. Availability of Ni as extracted with Mehlich-1 after 15-day incubation of soils (TQ: Typic Quartzipsamment and RH: Rhodic Hapludox) with increasing concentrations of Ni. ** Significant coefficient of the equation at 1% by *t*-test.

3.2. Nickel Effects on Shoot and Phytolith Yield in Grasses

The shoot dry matter yield of the grasses was affected by the Ni addition in both soils ($p < 0.01$). The square root model better explains the variation in the shoot dry matter of the grasses as a function of the Ni addition for each soil. The maximum dry matter yield of grasses was achieved with the maximum concentration (MC) of Ni added to the soils (Figure 2a,b), with an average MC of 30 mg kg^{-1} of Ni.

The grasses showed positive responses with increasing Ni concentrations added to the soil until reaching MC (Figure 2a,b), reflecting the essentiality of Ni as a micronutrient for plants. The positive response of grasses grown in the TQ soil was twice as high as when grown in the RH soil, with Ni concentrations added to the soil increasing from control (no Ni added) to reach MC (Table 2), due to greater Ni availability in the TQ soil (Figure 1). *M. maximus* had a response to Ni addition in the soil 2.4 times greater than *U. decumbens* when cultivated in the soils (TQ and RH) with increasing Ni concentrations from the control (without Ni addition) until reaching MC (Table 2). Therefore, the higher shoot dry matter yield of *M. maximus* compared to *U. decumbes* is due to its greater adaptability to different cultivation conditions.

The dry matter yield of grasses decreased after reaching the MC with increased Ni concentrations in the soils (Figure 2a,b), causing Ni toxicity due to increased Ni availability in soils (Figure 1). The Ni toxicity effect was 1.8 times higher in grasses grown in the TQ soil than in the RH soil (Table 2) due to higher Ni availability in the TQ soil (Figure 1). The comparison of grasses revealed that *M. maximus* had a shoot dry matter yield of 1.3 times greater compared to *U. decumbens,* with increasing concentrations of Ni added in the MC soil until the maximum concentration of added Ni (120 mg kg^{-1}) was reached. The greater tolerance potential of *M. maximus* to Ni can be attributed to the greater biomass production and its adaptability to the high availability of Ni in soils (Figure 1).

Phytolith yield by grasses was influenced by the increase in Ni concentrations ($p < 0.01$) only when grown in the TQ soil (Figure 2c,d). There was a reduction in phytoliths until MC for dry matter yield and a subsequent increase when the Ni concentrations increased, adjusting to the square root model (Figure 2c). The phytolith reduction is due to the dilution effect in the positive response of grasses to Ni, while the increase is due to the toxic effect of Ni in the shoots of grasses (Figure 2a). The dilution effect did not cause a difference in phytolith yield between the two grasses (Figure 2c). The effect of Ni toxicity caused a greater increase in the phytoliths in *U. decumbens* than in *M. maximus*, with a higher yield with the application of maximum concentration added (120 mg kg^{-1}) (Figure 2c), reflecting the genetic and physiological differences between grasses in the phytolith yield.

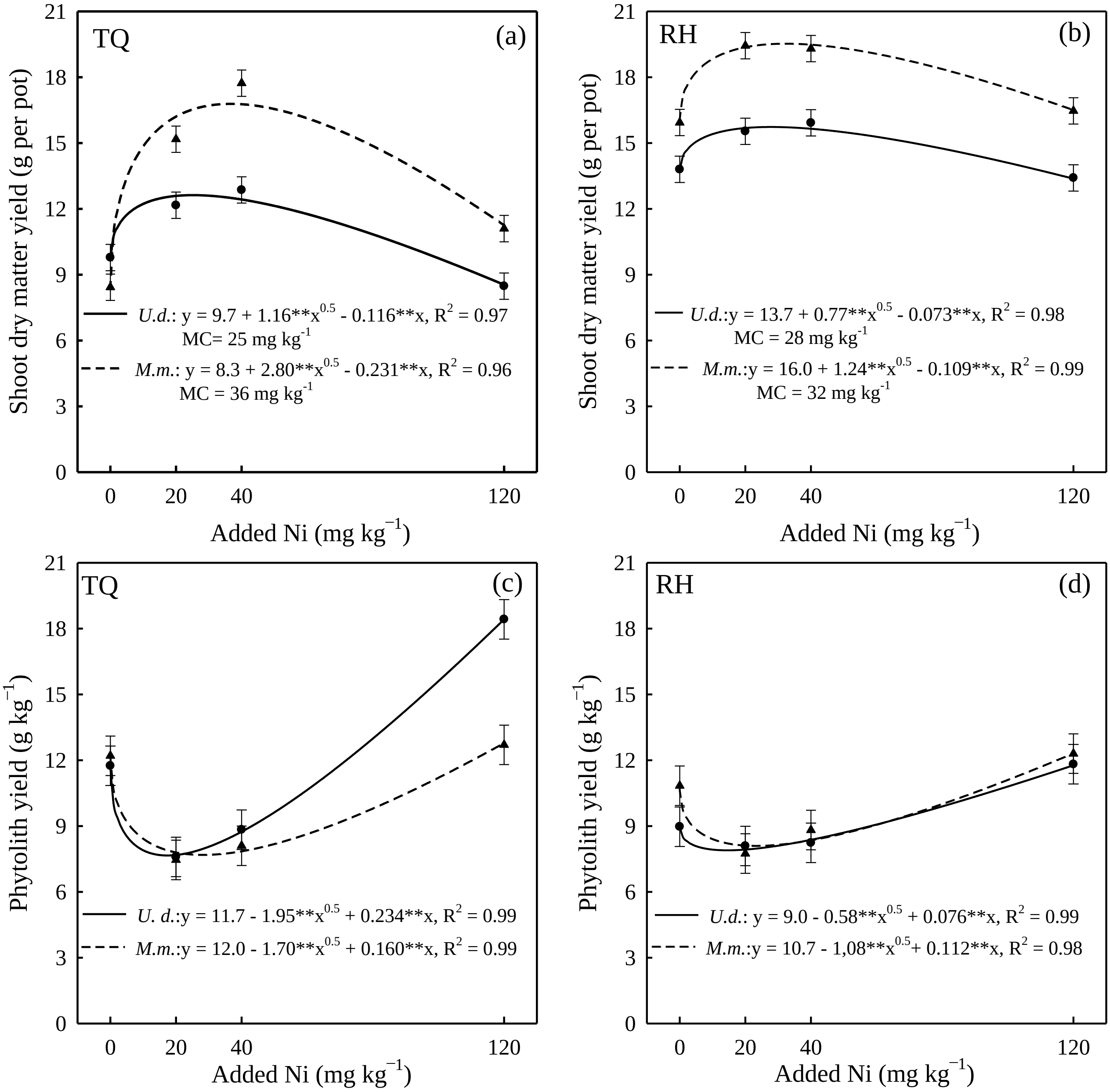

Figure 2. Shoot dry weight (**a**,**b**) and phytolith yield (**c**,**d**) of grasses (*Ud*: *Urochloa decumbens* and *M.m.*: *Megathyrsus maximus*) with increasing concentrations of Ni and maximum concentration (MC) within 120 days of thinning in two soils (TQ: Typic Quartzipsamment and RH: Rhodic Hapludox). ** Significant coefficient of the equation at 1% by *t*-test.

Table 2. Shoot dry matter yield corresponding to the control, maximum concentration (MC) for maximum shoot dry matter yield of two species of grasses, and maximum concentration added (MCA) to the soil within 120 days of thinning in two soils [(a)].

Grasses	Typic Qurtzipsmment (TQ)			Rhodic Hpludox (RH)		
	Control	MC	MCA	Control	MC	MCA
Urochlo decumens	9.7	12.6	8.5	13.7	15.7	13.4
Megthyrsus mximus	8.3	16.8	11.3	16.0	19.5	16.5
Average	9.0	14.7	9.9	14.9	17.6	15.0

[(a)] Shoot dry matter yield was estimated by replacing the control (no Ni addition), maximum concentration (MC) of shoot dry weight of two grasses, and maximum concentration added (MCA) to soils in the equations relating to the Ni concentrations (Figure 2a,b).

3.3. Nickel in Shoots and Phytoliths

Nickel concentrations in the shoot and phytoliths were assessed to examine Ni uptake and Ni sequestration within phytoliths by the grasses, respectively. The nickel concentrations in both shoots and phytoliths of grasses were significantly influenced by the Ni additions to the soils ($p < 0.01$). The addition of Ni into the soils exhibited a positive and linear correlation with the Ni concentrations in both the shoots and phytoliths of the grasses (Figure 3). Disparities in Ni uptake were observed among the grass species (Figure 3a,b), and Ni occlusion in phytoliths (Figure 3c,d) was evident only when cultivated in the TQ soil.

Elevated Ni concentrations in the shoots of grasses were observed when cultivated in the TQ soil compared to the RH soil (Figure 3a,b). This discrepancy is attributed to the greater Ni availability in the TQ soil (Figure 1), manifesting as an augmented positive response and a toxic impact of Ni on the dry matter yield of grasses (Figure 2a). Specifically, the Ni concentration in the shoot was higher in *Megathyrsus maximus* compared to *Urochloa decumbens* when cultivated in the TQ soil (Figure 3a).

The Ni occlusion within phytoliths exhibited a higher magnitude when grasses were grown in the TQ soil compared to the RH soil (Figure 3c,d). This response is attributed to the heightened toxic impact of Ni, stemming from its increased availability in the TQ soil (Figure 1). When the grasses reached their maximum shoot growth phase (Figure 2a), there was no discernible disparity in Ni occlusion (Figure 3c). This uniformity can be attributed to the adequacy of Ni concentration in the shoots to attain maximal growth, thereby affirming the essentiality of Ni as a micronutrient. Notably, *Urochloa decumbens* demonstrated a greater Ni occlusion in phytoliths than *Megathyrsus maximus* when cultivated in TQ soil at the highest applied concentration (120 mg kg^{-1}) (Figure 3c). This observation may be associated with the tolerance mechanism of *U. decumbens* to Ni toxicity.

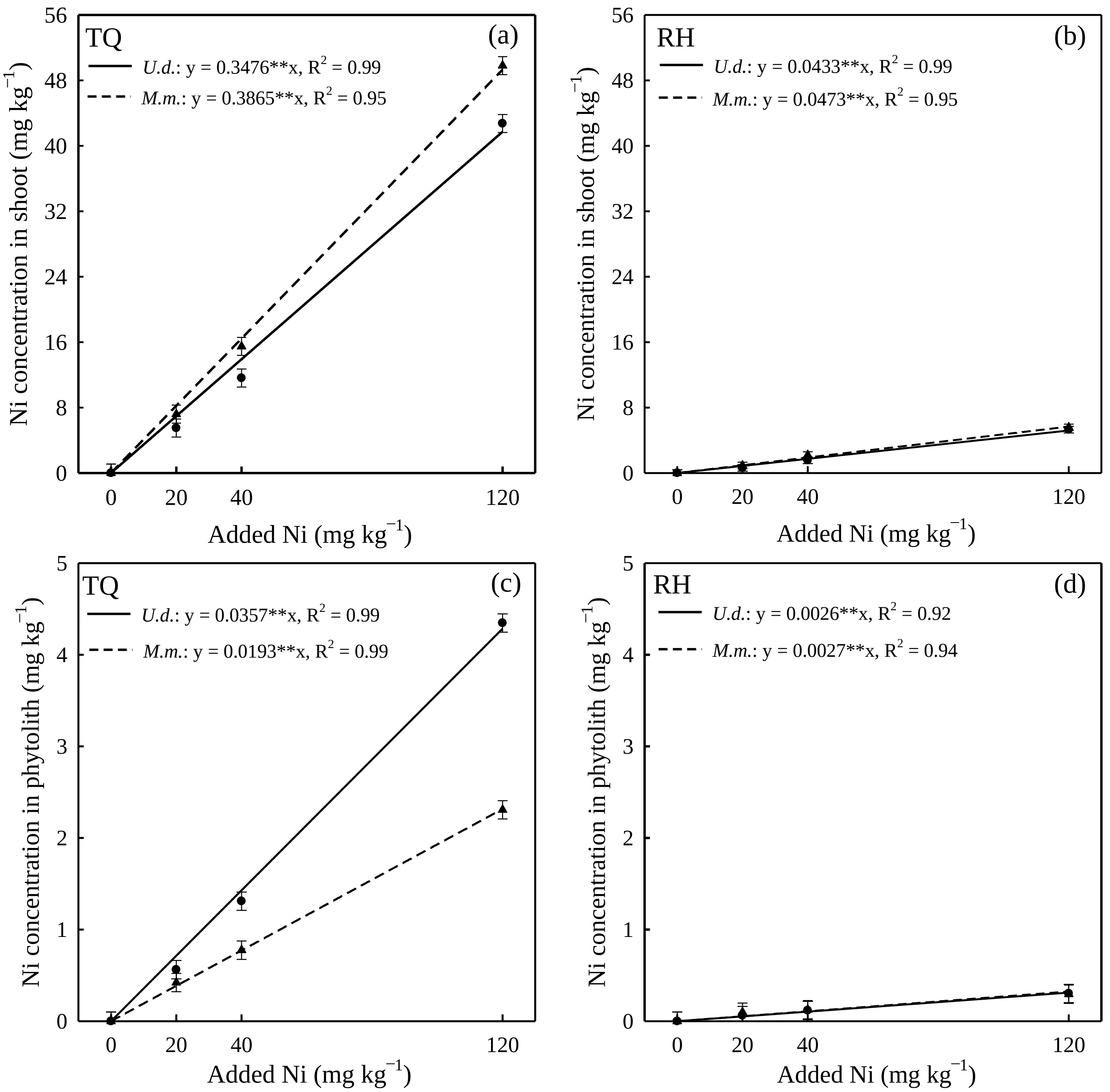

Figure 3. Concentration of Ni in the shoot (**a**,**b**) and phytolith (**c**,**d**) of grasses (*Ud*: *Urochloa decumbens* and *M.m.*: *Megathyrsus maximus*) with increasing concentrations of Ni within 120 days of thinning in two soils (TQ: Typic Quartzipsamment and RH: Rhodic Hapludox). ** Significant coefficient of the equation at 1% by *t*-test.

4. Discussion

The main differences between the evaluated soils that influenced the Ni availability for grasses were the concentrations of iron and aluminum oxides, carbon organic, and clay (Table 1). Such differences in soil characteristics resulted in a lower CEC for the TQ soil when compared to the RH soil (Table 1). Consequently, Ni became more available in the TQ soil (Figure 1), providing increased Ni uptake and Ni occlusion in the phytoliths by the grasses (Figure 3a,c) and causing an increase when reaching the MC of Ni and, subsequently, a reduction in the biomass yield (Figure 2a) and, inversely, in the phytolith yield (Figure 2c). Ni retention occurs, for the most part, through the electrostatic forces of the negatively charged particles, which makes it highly dependent on soil CEC [5,7,13,14].

The interaction between chemical forms of metals present in the soil solution and the surface of clay minerals and organic colloids in the soil, called adsorption, is the chemical process that most alters the metal availability in the soil [13,14].

The yield of plants grown in contaminated soil acts as a bioindicator of a plant's response to elements added to the soil. The maximum concentrations (MC) of Ni (Figure 2) can be considered as recommended concentrations of Ni for the growth of grasses in soils. The recommended average concentration of 30 mg kg^{-1} of Ni is in the range commonly found in agricultural soils [13] and reinforces the essentiality of Ni for plants [1,2]. On the other hand, the MC of Ni (Figure 2) was below the investigation value (70 mg kg^{-1} of Ni) established by Brazilian legislation for agricultural soils [12]. Therefore, unacceptable Ni concentrations for agricultural soils were toxic to grasses even in pot experiments, showing grass sensitivity to excess Ni in soils [1,2].

The grasses responded differently to Ni application (Figure 2a,b), proving Ni's essentiality as a micronutrient, i.e., low concentrations of Ni have a significant effect on plant growth and development. When the MC was reached, it reduced the shoot dry matter of the grasses, reflecting the toxicity effect on the plants. The supply of Ni in low concentrations increased the shoot dry matter yield of the grasses up to the MC, while higher concentrations added to the soil above the MC reduced the dry matter of the grasses in both soils (Figure 2a,b). The results prove the essentiality of Ni as a micronutrient [1,2]; that is, in small concentrations Ni has a positive effect, increasing the growth of grasses, but it has a toxic effect on plants in high concentrations above the MC, resulting in lower grass growth [1,14]. The decline in grass growth following exposure to Ni after reaching the MC also corresponds to the inhibition of active enzymes, mineral nutrition, and chlorophyll biosynthesis in plants [5].

M. maximus responded most and tolerated to soil Ni, i.e., it presented higher growth and less reduction in the shoot when compared to *U. decumbens* (Figure 2a,b). The *Megathyrsus maximus* species has a high productive potential in relation to the genus *Urochloa*, being more responsive to soil fertilization and adaptation to different soil conditions [1,10]. The species *M. maximus* was more responsive when grown in the TQ soil than in the RH soil compared to *U. decumbens* (Figure 2a,b). This suggests that in addition to the species, the texture and mineralogy of the soil influence the availability of Ni and, consequently, the absorption of Ni by the plant. Ni absorption by plants depends on the metabolism and plant species [1,10], the form of Ni and concentration in the soil [1,2,5], the presence of other nutrients [5], and the physical and chemical attributes of the soil [7,13,14].

The phytolith yield of grasses and Ni toxicity in the shoot in response to Ni application in soils (Figure 2a,b) provide the potential of grasses as producers of phytoliths (Figure 2c,d). The reduction in phytolith yield from grasses (Figure 2c,d) in response to the Ni application from the shoot (Figure 2a,b) is caused by the phytolith's dilution effect on the plant. The effect can be characterized when the relative growth rate of the shoot is higher than the relative yield rate of the phytolith. The superior growth of the shoot of grasses in response to Ni application demonstrates the essentiality of Ni for plants [1,2]. However, under conditions of stress caused by excess Ni, the grasses showed an increase in phytolith yield when cultivated in TQ soil (Figure 2c). The greater formation of phytoliths in conditions of excess Ni suggests the presence of a detoxification mechanism of grasses to Ni. The role of phytoliths as a plant detoxification mechanism for various heavy metals has been reported in some studies [6,8,9].

The grasses had different capacities for producing phytoliths in conditions of Ni toxicity in soil with greater availability of Ni, such as TQ soil (Figure 2c). The phytolith concentrations in plants are correlated with the Ni availability in the soil [7,15], which can directly reflect the phytolith yield of grasses [6–9]. The greatest increase in phytolith yield occurred with *U. decumbens*, rather than *M. maximus,* in response to the toxic effect of Ni (Figure 2c), where the greater rusticity of the genus *Urochloa* [11] possibly provided greater phytolith yield to tolerate Ni toxicity. However, the reasons for the phytolith yield of plants are still unknown, but phytoliths can be produced in adverse conditions and can increase the resistance of many terrestrial plants against biotic and abiotic stress, especially species of the Cyperaceae and Poaceae families [6,9], which are the families of the grasses evaluated [11].

The Ni concentration in the shoot and phytoliths was inverse between the grasses *U. decumbens* and *M. maximus* when cultivated in TQ soil (Figure 3a). This result is contrary to that observed in rice, where it was observed that the higher the elemental concentration of the plant, the higher the metal concentration in the phytoliths [7], demonstrating that *U. decumbens* develops a mechanism that allows the capture and stabilization of Ni in phytoliths. This capture is possibly due to the higher rusticity and ability to grow in adverse soil conditions in relation to *Megathyrsus maximus* [11]. The phytolith yield is a mechanism of tolerance to the metal developed by the plant, which immobilizes and makes the metal unavailable, giving phytoliths the characteristic of sequestering the metal and preventing its return to the soil in cases of senescence of the plant or ingestion by fauna, making the metal unavailable to the food chain [8]. The correlation between the concentration of phytoliths in plants and the elemental concentration of phytoliths, that is, the greater the availability of the element in the soil, the greater its absorption, significantly affecting the production of phytoliths in plant organs [7]. However, the reasons for phytoliths' yield by plants are still poorly understood, but it is known that phytoliths can be produced under adverse conditions and increase resistance against biotic and abiotic stress in many land plants, especially species from the Cyperaceae family and Poaceae [6,9]. Several factors can influence these phytoliths' yield by plants, such as taxonomic character, environmental conditions, availability of water in the soil, availability of monosilic acid in the soil solution, and evapotranspiration rate [6,8,9]. Furthermore, the morphology and chemical composition of phytoliths and environmental conditions can affect the sequestration of elements in phytoliths [6,8,9]. This explains why the elemental composition in organs is related to the elemental composition in phytoliths [7].

The toxicity level of Ni patterns in plant species is classified into sensitive, moderately tolerant, and non-tolerant [5]. *U. decumbens* is classified as a sensitive species because it has Ni toxicity when the concentration in the shoot is less than 10 mg kg^{-1} e. *M. maximus* is moderately tolerant because it has a Ni concentration between 10 and 50 mg kg^{-1} (Figure 3a). Therefore, the increased yield (Figure 2c) and Ni occlusion (Figure 3c) in phytoliths by *U. decumbens* did not confer greater tolerance to Ni toxicity compared to *M. maximus*. In contrast, *M. maximus* presented desirable characteristics such as a higher dry matter yield (Figure 2a) and shoot Ni concentration (Figure 3a), indicating that this species is more tolerant to excess Ni. Grasses with phytoremediation potential should have desirable phytoextraction characteristics, such as high growth rate, biomass yield, root growth, and the ability to tolerate and accumulate toxic metals [3,4].

The *M. maximus* grass had the highest growth response, accumulation of Ni in the shoot, and less yield and Ni occlusion in phytoliths to *U. decumbens* (Figure 3), demonstrating that the yield and Ni sequestration by phytoliths is not a possible mechanism of tolerance to Ni toxicity but has desirable characteristics of grasses with phytoremediation potential [3,4]. Some researchers claim that it is possible to improve the rate of metal sequestration in phytoliths through the selection of high-production species and the sequestration of elements in the phytolith [15]. However, species cannot be selected solely based on yield and metal sequestration by the phytolith but must be considered in combination with other desirable characteristics, such as biomass production and productivity [15]. In addition, other limiting factors, such as differences in location, climatic conditions [15], and species characteristics, need to be considered, as well as other mechanisms involved in plants' tolerance to heavy metals [3].

5. Conclusions

Nickel was phytotoxic to grasses at an added concentration of about 30 mg kg^{-1} below the investigation value (70 mg kg^{-1}) for agricultural soils established by Brazilian Environmental Legislation.

The yield and Ni occlusion in phytoliths by grasses in Ni-contaminated soils is related to the genetic and physiological differences between grasses and the Ni availability in soils.

Ni capture by phytoliths indicates that it may be one of the detoxification mechanisms of *Urochloa decumbens* to Ni-contaminated soils, providing additional evidence that *Megathyrsus maximus* may be a future grass for the phytoremediation technique for Ni-contaminated soils.

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