

Changes caused by heavy metals in micronutrient content and antioxidant system of forage grasses used for phytoremediation: an overview

Alterações causadas por metais pesados na concentração de micronutrientes e no sistema antioxidante de gramíneas forrageiras usadas para fitorremediação: uma visão global

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— REVIEW —

ABSTRACT

An increase in the content of heavy metals in the environment causes many socio-environmental problems, and phytoremediation is a tool to reduce the environmental impact caused by these elements, with prospects for the use of forage grasses. This group of plants features characteristics for the environment-decontamination process, but further studies are necessary about the damages caused by heavy metals on the uptake of cationic micronutrients and on the antioxidant system, which are essential processes for the growth of plants in contaminated sites. Exposure of forage grasses to heavy metals results in a lower content of Mn in the shoots of almost all plants, but the contents of Cu, Fe, and Zn vary according to heavy metal and forage grass. Activities of enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GPX) usually increase to reduce the oxidative stress induced by heavy metals, but when the content of any of these metals is high, enzymatic activity is decreased. Scale of toxicity of heavy metals to forage grasses can be described as: $Pb \approx Cr > Cd \approx As > Zn \approx Cu \approx Ni > Mn$.

Key words: biomass, nutritional status, oxidative stress, environmental pollution.

RESUMO

O aumento da concentração de metais pesados no ambiente provoca muitos problemas sócioambientais, de forma que a fitorremediação é uma ferramenta para diminuir o impacto ambiental causado por metais pesados, com perspectivas para o uso de gramíneas forrageiras. Esse grupo de plantas apresenta características desejáveis para o processo de descontaminação do ambiente, mas é necessária a realização de mais estudos acerca dos danos causados por metais pesados na absorção de micronutrientes catiónicos e no sistema antioxidante, que são processos fundamentais para o crescimento de plantas em locais contaminados. A exposição das gramíneas forrageiras aos metais

pesados diminui a concentração de Mn na parte aérea de quase todas as plantas, mas as concentrações de Cu, Fe e Zn variam em função do metal pesado e da gramínea. As atividades das enzimas superóxido dismutase (SOD), ascorbato peroxidase (APX), catalase (CAT) e guaiacol peroxidase (GPX) quase sempre aumentam para diminuir o estresse oxidativo induzido pelos metais pesados, mas, quando a concentração do metal é alta, a atividade enzimática diminui. A escala de toxicidade dos metais pesados para as gramíneas forrageiras pode ser descrita como: $Pb \approx Cr > Cd \approx As > Zn \approx Cu \approx Ni > Mn$.

Palavras-chave: biomassa, estado nutricional, estresse oxidativo, poluição ambiental.

INTRODUCTION

The content of heavy metals¹ in the environment has increased considerably in recent years due to the improper discard of industrial waste, to intensification of agricultural activities, and to the expansion of urban areas, resulting in serious socio-environmental problems (ZALEWSKA, 2012). In most cases, uptake of metals causes a reduction of biomass production by plants, but in extreme cases in which the content of these metals is too high, the area may become desertified (KOPTSIK, 2014). This situation can also lead to biodiversity decline by the selection of the most resistant plants (ZHANG et al., 2014a). In addition, cultivation of plants in contaminated areas promotes the uptake of heavy metals, which, when consumed by humans, may

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cause numerous health problems, such as emphysema, gastric dysfunction, and cancer (ZALEWSKA, 2012; LOU et al., 2015).

The presence of heavy metals in soil solution or in nutrient solution firstly modifies the uptake of nutrients by plants, especially of cationic micronutrients Cu^{2+} , Fe^{2+} , Mn^{2+} , and Zn^{2+} due to their competition for the same uptake sites located in the roots (ZHANG et al., 2014a,b). After they are absorbed, heavy metals can alter the process of transfer of electrons existing in cell organelles, providing favorable conditions to the generation of reactive-oxygen species (ROS), which cause lipid peroxidation of membranes² in all plant tissues (FARMER & MUELLER, 2013). Alteration in the process of uptake of micronutrients associated with the generation of ROS, such as hydroxyl (OH^\cdot), superoxide (O_2^\cdot), and singlet oxygen ($^1\text{O}_2$), which are the most reactive ROS present in the cell medium, are within the main factors resulting in decreased biomass production by plants (GILL & TUTEJA, 2010; ZHANG et al., 2014a,b). Thus, it is essential that plants exposed to heavy metals have an efficient antioxidant system so as to lessen the injuries caused by the ROS (GRATÃO et al., 2005; LUO et al., 2011). Plant antioxidant system is composed of non-enzymatic and enzymatic antioxidants, and enzymes superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT) and ascorbate peroxidase (APX) are among the main enzymes involved in the ROS-elimination process (GILL & TUTEJA, 2010). Consequently, the activity of these enzymes can be used to identify plants presenting efficient antioxidant systems to be used in environments contaminated by heavy metals (LI et al., 2012). The use of plants able to uptake and accumulate large amounts of heavy metals (hyperaccumulator plants) in short periods is a very promising alternative to reduce the content of heavy metals and mitigate the damage caused by these elements in contaminated environments, although these plants usually have low biomass production (KOPTSIK, 2014).

In this way, the use of forage grasses for phytoremediation³ of heavy metals has increased considerably in recent years, as these plants show rapid growth, an extensive root system (which increases the uptake of heavy metals), and elevated dry mass production (CHEN et al., 2012; GILABEL et al., 2014; LAMBRECHTS et al., 2014). However, few studies with these plants have reported the effect of heavy metals on the uptake of micronutrients and on the activity of enzymes of the antioxidant system, which are essential processes for the growth

of plants, especially in contaminated environments (KOPITTKKE et al., 2010a; LI et al., 2012). Therefore, the objective of this review was to identify the alterations caused by distinct heavy metals in the content of cationic micronutrients, in the activity of antioxidant enzymes, and in the biomass production of forage grass, and to evaluate the implication of these alterations for phytoremediation.

Importance of cationic micronutrients for plant growth

The Cu, Fe, Mn, and Zn are essential for the normal growth of plants because of the roles they play. Copper participates in the electron transport in photosystems I and II and in the fixation of N_2 , among other functions. Iron is a component of ferredoxin and is involved in the reduction of nitrate (NO_3^-) and sulfate (SO_4^{2-}), as well as in energy production (NADP). Manganese participates in the photolysis of water and is involved in redox processes in the transport system of electrons of photosynthesis. Zinc participates in enzymes that work in the fixation of C and in the synthesis of proteins, among other activities (MARSCHNER, 2012). In view of these functions, it is evident that the alterations brought about by heavy metals in the Cu, Fe, Mn, and Zn uptake will have an impact on plant growth (BONNET et al., 2000).

Relationship between heavy metals and cationic micronutrients in forage grasses

At first, it is observed that the alteration in the content of the micronutrients Cu, Fe, and Zn in the tissues of the shoots of forage grasses is dependent on the studied heavy metals and plant species (Table 1). It can be thus noted that the concentrations of the cationic micronutrients of the *Brachiaria decumbens* (Syn. *Urochloa decumbens*) exposed to Cd decreased (KOPITTKKE et al., 2010a; TOLENTINO et al., 2014), whereas the concentrations of Fe and Zn increased in the cultivation of *Panicum maximum* (RABÊLO, 2014) and *Pennisetum americanum* × *P. purpureum* (ZHANG et al., 2014b) exposed to Cd. Therefore, this alteration depends on the tolerance of plants to the heavy metal and on the expression of genes that code proteins specialized in transporting heavy metals (nutrients or not) from roots to shoots (LUX et al., 2011); this suggests that *Panicum maximum* and *Pennisetum americanum* × *P. purpureum* are more tolerant⁴ to the damage caused by the metals than *Brachiaria decumbens*. This tolerance can be attributed to the greater detoxification capacity of metals (e.g., higher synthesis of glutathione - GSH,

phytochelatins and amino acids, and high activity of the antioxidant system) (GRATÃO et al., 2005; NOCTOR et al., 2011).

Despite the variation observed in the content of the cationic micronutrients Cu, Fe, and Zn in the shoots of the forage grasses grown in the presence of heavy metals, the content of Mn decreased in almost all situations (Table 1). This result occurs because the main mechanism of ion-root contact⁵ by Mn is root interception, i.e., the lower root growth observed in conditions with supply of heavy metals limits the uptake of Mn and consequently the content of the micronutrient in the shoots of the plants is reduced (KOPITKE et al., 2007, 2010a,c; MARSCHNER, 2012). This fact; however, is apparently not a problem for the use of these plants in phytoremediation processes, since they develop normally within a broad range of Mn concentrations in the shoots that varies from 40 to 200mg kg⁻¹ dry matter (WERNER et al., 1996). However, the adequate content ranges

of Cu (4-12mg kg⁻¹ dry matter) and Zn (20-50mg kg⁻¹ dry mass) for the growth of forage grasses are narrow when compared with the range of Mn (WERNER et al., 1996). In this regard, forage grasses whose contents of Cu and Zn are reduced when cultivated in the presence of toxic elements like Cd and Pb may have their growth considerably limited and be inefficient if used in the process of phytoremediation of environments with extremely high contents of heavy metals. It should be mentioned that Cu and Zn are components of metalloenzymes⁶ and are involved in oxidation-reduction processes, in detoxification of O₂⁻, and in protein and lignin syntheses, i.e., in processes essential to plants exposed to heavy metals (MARSCHNER, 2012; RIBERA-FONSECA et al., 2013). Regarding Fe, the appropriate content range for forage grasses (50-250mg kg⁻¹ dry matter) is wider than the ranges of Cu and Zn, but a decrease in the content of this nutrient affects the photosynthesis (WERNER et al., 1996; MARSCHNER, 2012).

Table 1 - Effects of heavy metals on the micronutrient content and activity of antioxidant enzymes in forage grasses.

Plant species	GC ¹	Rate ⁴	HM ⁵	Cu	Fe	Mn	Zn	Reference
<i>Avena sativa</i>	soil	39.95	Ni	↑	↑	↓	-	DAN et al. (2008)
<i>Brachiaria decumbens</i>	NS ²	1.0	Cd	↓	↓	↓	↓	TOLENTINO et al. (2014)
<i>Brachiaria decumbens</i>	NS	-	Cd	-	-	↓	-	KOPITKE et al. (2010a)
<i>Brachiaria decumbens</i>	NS	-	Ni	-	-	↓	-	KOPITKE et al. (2010c)
<i>Brachiaria decumbens</i>	NS	-	Ni	-	↓	-	-	KOPITKE et al. (2008)
<i>Brachiaria decumbens</i>	NS	-	Pb	↓	↓	↓	↓	KOPITKE et al. (2007)
Hybrid ³	soil	0.88	Cd	↑	-	↓	↑	ZHANG et al. (2014a)
Hybrid ³	soil	0.88	Cd	↑	↑	↓	↑	ZHANG et al. (2014b)
<i>Lolium perenne</i>	soil	0.44	Cd	-	-	-	↑	LAMBRECHTS et al. (2014)
<i>Lolium perenne</i>	NS	20.00	Zn	-	↓	↑	↑	MONNET et al. (2005)
<i>Panicum maximum</i>	NS	2.00	Cd	-	↑ ⁶	↓ ⁶	↑ ⁶	RABÊLO (2014)
<i>Panicum mosambicense</i>	NS	-	Cu	↓	↓	↓	↓	KOPITKE et al. (2009)
Plant species	GC ¹	Rate ⁴	HM ⁵	APX ⁷	CAT ⁸	GPX ⁹	SOD ¹⁰	Reference
<i>Avena sativa</i>	NS	1.37	Cd	-	-	↑	-	ASTOLFI et al. (2004)
<i>Avena strigosa</i>	NS	0.01	Cd	↓	-	-	↑	URAGUCHI et al. (2009)
<i>Festuca arundinacea</i>	soil	3.06	Ni	↓	↑	↑	↓	MIRZAHOSSEINI et al. (2014)
<i>Festuca arundinacea</i>	NS	4.82	Pb	↑	-	↑	-	HU et al. (2015)
<i>Lolium perenne</i>	NS	0.50	Cd	↑	↑	↑	↑	LUO et al. (2011)
<i>Lolium perenne</i>	NS	0.50	Cd	-	↑	-	↑	LOU et al. (2015)
<i>Lolium perenne</i>	NS	0.75	Mn	-	-	-	↑	RIBERA-FONSECA et al. (2013)
<i>Lolium perenne</i>	NS	3.20	Pb	-	↓	-	↓	LI et al. (2012)
<i>Lolium perenne</i>	NS	50.00	Zn	↑	-	-	↑	BONNET et al. (2000)
<i>Panicum maximum</i>	NS	2.00	Cd	↑ ⁶	-	-	-	RABÊLO (2014)
<i>Vetiveria zizanoides</i>	soil	0.35	Cd	-	↑	-	↑	WEIHONG et al. (2009)
<i>Vetiveria zizanoides</i>	soil	1.22	Zn	-	↑	-	↑	WEIHONG et al. (2009)

¹GC = growth conditions. ²NS = nutrient solution. ³*Pennisetum americanum* × *P. purpureum*. ⁴mmol L⁻¹ for nutrient solution or mmol kg⁻¹ for soil. ⁵HM = heavy metal. ⁶Results obtained in the second grass growth period. ⁷APX - ascorbate peroxidase. ⁸CAT - catalase. ⁹GPX - guaiacol peroxidase. ¹⁰SOD - superoxide dismutase.

Interrelationship between heavy metals and the antioxidant system of forage grasses

Plants normally produce ROS because of their photo-respiratory nature, but under normal growth conditions, there is a balance between the production of ROS and antioxidants (GILL & TUTEJA, 2010). In the presence of heavy metals, this balance is broken, with the ROS (oxidative stress) predominating in the cell medium, which may result in damages to proteins, lipid, carbohydrates, DNA, and consequently cell death (GRATÃO et al., 2005; FARMER & MUELLER, 2013). Magnitude of damages varies according to the heavy metal and tolerance of plant species (WEIHONG et al., 2009). This occurs because some heavy metals are more toxic to plants than others, due to components to which they bind (metals that bind to O-rich compounds are less toxic than metals that bind to N-rich compounds) and to the molecular processes in which they participate (production of reactive species by autoxidation, displacement of essential metal ions to biomolecules, and blocking of essential functional groups), with Pb and Hg normally being more toxic than Ni and Zn for most plants (NIEBOER & RICHARDSON, 1980; SCHUTZENDUBEL & POLLE, 2002; KOPITTKÉ et al., 2010b,c). For instance (Table 1), the activities of enzymes APX, CAT, GPX, and SOD from plants exposed to Zn increased, but the activities of enzymes CAT and SOD of forage grasses exposed to Pb were reduced (BONNET et al., 2000; WEIHONG et al., 2009; LI et al., 2012). This fact demonstrates that the generation of ROS and the inactivation of enzymes in plants exposed to Pb are greater than in plants exposed to Zn (HU et al., 2015).

In most cases, the activity of enzymes from the antioxidant system increases as an attempt to combat the ROS, but when the content of the heavy metal in the plant tissue or the generation of ROS is too high, enzymatic activity may be reduced (GRATÃO et al., 2005; HU et al., 2015). The lower enzymatic activity may be caused by the oxidation of the thiol groups⁷ from the enzymes due to the increase in the generation of hydrogen peroxide (H₂O₂) (GILL & TUTEJA, 2010). In *Avena strigosa* plants exposed to Cd, activity of SOD increased, while the CAT activity declined (Table 1). This may indicate that SOD was efficient in the dismutation of O₂⁻, but H₂O₂ generated in the dismutation of O₂⁻ deactivated CAT. It is noteworthy that SOD acts in the dismutation of O₂⁻ radicals; whereas CAT, GPX, and APX act in the detoxification of H₂O₂ (GILL & TUTEJA, 2010). Thus, it is essential that plants established in environments contaminated by heavy metals have an efficient antioxidant system

(GRATÃO et al., 2005; HU et al., 2015). Forage grasses have distinct degrees of tolerance to damages caused by heavy metals, and increased activity of antioxidant enzymes can be considered a desirable evolutionary process for the use of forage grasses in the phytoremediation process (WEIHONG et al., 2009; KOPTSIK, 2014; MIRZAHOSSEINI et al., 2014; HU et al., 2015; LOU et al., 2015).

The results displayed in table 1 showed that there is a clear interaction between the heavy metals and the species of forage grasses that can change the efficiency of the antioxidant system in plants (URAGUCHI et al., 2009; LI et al., 2012; MIRZAHOSSEINI et al., 2014). However, few studies have been conducted on this matter, especially considering that the alteration of the antioxidant system is also associated with the uptake of cationic micronutrients, since isoforms⁸ of SOD (Cu/Zn-SOD, Fe-SOD, and Mn-SOD) are also activated by the enzyme cofactors Cu, Fe, Mn, and Zn (GRATÃO et al., 2005; GILL & TUTEJA, 2010). As in the case of SOD, there are also isoforms of CAT, APX, and GPX (GILL & TUTEJA, 2010). Transcription of genes that code SOD isoforms in *Lolium perenne* plants increased when they were exposed to Pb (LI et al., 2012) and to Mn (RIBERA-FONSECA et al., 2013). However, in *Panicum maximum* grown with Cd, no alterations were identified in SOD and CAT isoforms (RABÊLO, 2014). These results demonstrated once again the specificity between heavy metals and plant species, indicating the need for research on this subject to identify the responses of the antioxidant system of forage grasses cultivated under the presence of heavy metals (nutrients or not).

Effect of heavy metals on biomass production and implications for phytoremediation

In addition to the alterations in the nutrient uptake process and in the antioxidant system of forage grasses, other damages can be caused by heavy metals, such as alterations in the metabolism of amino acids, in the apparatus of the photosynthetic system, in syntheses of chlorophyll and carotenoids, and in cell structure (LUO et al., 2011; LOU et al., 2015). Moreover, the damages caused depend on the toxicity of the heavy metal and on the plant's ability to tolerate this toxicity (NIEBOER & RICHARDSON, 1980; SCHUTZENDUBEL & POLLE, 2002; ZHANG et al., 2014b). Toxicity caused by the heavy metal, in turn, is dependent on the available concentration of this metal in the soil or in the nutrient solution, which can be changed by t pH and by organic matter content of the medium, among other factors (ZENG et al., 2011). In general, heavy metals have a negative correlation with the pH of the medium and with the organic matter content,

such that the availability of these elements is reduced with an increase in pH and organic matter (ZENG et al., 2011; MARSCHNER, 2012). Table 2 shows that all the studied grasses had a reduction in biomass production, irrespectively of the heavy metal supplied; however, the lowest reductions of biomass occurred in conditions of higher pH and higher organic matter content.

It should be emphasized that in addition to the afore-mentioned factors (pH and organic matter content) that control the bioavailability of the heavy metal in the solution of the medium, some factors inherent to the heavy metal (e.g., compounds to which they bind and

molecular processes in which they participate) and the plant (e.g., low concentration of GSH) may result in lower biomass production (KOPTSIK, 2014). As can be seen in table 2, exposure of plants of the genus *Avena* to Cr and Pb reduced biomass production by almost 100%, i.e., there was practically no plant growth (ANDRADE et al., 2009; WYSZKOWSKI & RADZIEMSKA, 2013). These two elements deactivated enzymes linked to the synthesis or restitution of GSH, which is considered the most active non-enzymatic antioxidant in plants exposed to metals, decreasing biomass production by plants (NOCTOR et al., 2011).

Table 2 - Effects of heavy metals on biomass production by forage grasses.

Plant species	GC ¹	pH ⁷	OM ⁹	HM ¹⁰	Rate ¹¹	Biomass	Reference
<i>Avena sativa</i>	NS ²	-		Cd	1.37	↓ 30%	ASTOLFI et al. (2004)
<i>Avena sativa</i>	soil	7.6	25.5	Cd	1.37	↓ 45%	ASTOLFI et al. (2011)
<i>Avena sativa</i>	soil	4.5	13.5	Cr	2.88	↓ 98%	WYSZKOWSKI & RADZIEMSKA (2013)
<i>Avena sativa</i>	soil ³	6.5	275.8	Ni	39.95	↓ 71%	DAN et al. (2008)
<i>Avena strigosa</i>	NS	5.1		Cd	0.01	↓ 30%	URAGUCHI et al. (2009)
<i>Avena strigosa</i>	soil ⁴	7.6	25.5	Pb	-	↓ 98%	ANDRADE et al. (2009)
<i>Brachiaria arrecta</i>	NS	5.0		As	0.05	↓ 39%	ARGENTA et al. (2013)
<i>Brachiaria decumbens</i>	NS	4.2		Ni	-	↓ 50%	KOPITTKKE et al. (2008)
<i>Brachiaria decumbens</i>	NS	4.2		Cd	-	↓ 50%	KOPITTKKE et al. (2010a)
<i>Brachiaria decumbens</i>	NS	4.2		Pb	-	↓ 50%	KOPITTKKE et al. (2007)
<i>Festuca arundinacea</i>	NS	5.5		Cd	0.17	↓ 82%	SOLEIMANI et al. (2010)
<i>Festuca arundinacea</i>	soil	-	-	Ni	3.06	↓ 59%	MIRZAHOSSEINI et al. (2014)
<i>Festuca pratensis</i>	NS	5.5		Cd	0.17	↓ 73%	SOLEIMANI et al. (2010)
Hybrid ⁵	soil	6.0	39.0	Cd	0.88	↓ 85%	ZHANG et al. (2014a)
Hybrid ⁵	soil	6.0	39.0	Cd	0.88	↓ 46%	ZHANG et al. (2014b)
<i>Lolium perenne</i>	soil	-	-	Cd	0.44	↓ 57%	LAMBRECHTS et al. (2014)
<i>Lolium perenne</i>	NS	-		Zn	50.00	↓ 86%	BONNET et al. (2000)
<i>Lolium perenne</i>	soil	-	-	Zn	1.50	↓ 64%	LAMBRECHTS et al. (2014)
<i>Lolium perenne</i>	NS	-		Zn	20.00	↓ 52%	MONNET et al. (2005)
<i>Lolium perenne</i>	soil ⁶	5.7 ⁸	9.7	Zn	6.11	↓ 31%	ZALEWSKA (2012)
<i>Panicum aquaticum</i>	NS	-		As	2.00	↓ 87%	PIRES et al. (2013)
<i>Panicum maximum</i>	NS	-		Cd	1.86	↓ 65% ¹²	RABÊLO (2014)
<i>Panicum maximum</i>	NS	-		Cu	1.00	↓ 38% ¹³	GILABEL et al. (2014)
<i>Panicum mosambicense</i>	NS	4.2		Cu	-	↓ 50%	KOPITTKKE et al. (2009)
<i>Panicum mosambicense</i>	NS	4.2		Ni	-	↓ 50%	KOPITTKKE et al. (2010c)
<i>Panicum virgatum</i>	NS	5.0		Cd	0.02	↓ 82%	CHEN et al. (2012)
<i>Panicum virgatum</i>	NS	5.0		Cr	0.04	↓ 55%	CHEN et al. (2012)
<i>Panicum virgatum</i>	NS	5.0		Zn	0.45	↓ 89%	CHEN et al. (2012)
<i>Paspalum notatum</i>	soil ⁴	7.6	25.5	Pb	-	↓ 92%	ANDRADE et al. (2009)
<i>Paspalum notatum</i>	NS	-		Zn	1.50	↓ 73%	SCHUERGER et al. (2003)
<i>Sorghum bicolor</i>	soil	7.0	-	Zn	32.11	↓ 59%	MIRSHEKALI et al. (2012)
<i>Sorghum sudanense</i>	NS	5.0		Cu	0.05	↓ 22%	WEI et al. (2008)
<i>Vetiveria zizanoides</i>	soil	7.8	11.8	Cd	0.35	↓ 14%	WEIHONG et al. (2009)
<i>Vetiveria zizanoides</i>	soil	7.8	11.8	Zn	1.22	↓ 14%	WEIHONG et al. (2009)

¹GC = growth conditions. ²NS = nutrient solution. ³Dates of Till Clay soil. ⁴Dates of soil 4. ⁵*Pennisetum americanum* × *P. purpureum*. ⁶Dates of sand soil. ⁷pH in CaCl₂. ⁸pH in KCl. ⁹OM = organic matter (g kg⁻¹ soil). ¹⁰HM = heavy metal. ¹¹mmol L⁻¹ for nutrient solution or mmol kg⁻¹ for soil. ¹²Result obtained from the second growth period. ¹³Result obtained from the grass growth period.

Exposure of some genera of forage grasses to Cd and As caused reductions in dry mass production of over 80% (SOLEIMANI et al., 2010; CHEN et al., 2012; PIRES et al., 2013; ZHANG et al., 2014a). These metals also deactivate enzymes bound to the synthesis or restoration of GSH, but for some plants groups this occurs less effectively compared with Cr and Pb (NOCTOR et al., 2011). However, some species of forage grasses showed a less than 50% decrease in biomass production when exposed to Cd (Table 2). This result demonstrates that besides the factors controlling the bioavailability of heavy metals, some genera of forage grasses are more tolerant than others, and the use of the most tolerant grasses for the phytoremediation of heavy metals is viable in the current scenario (ZHANG et al., 2014a, b).

Comparatively, the exposure of the forage grasses to Cu, Mn, Ni, and Zn caused less damage than Pb, Cr, Cd, and As, if it is considered that the supplied levels of these micronutrients were higher than those of the toxic elements (Table 2). Thus, it should be stressed that plants that produce more biomass allow the extraction of heavy metals in contaminated environments to occur more efficiently, because the uptake of the contaminant is higher, and consequently the decontamination of the environment is faster. Reduction of biomass production by plants increases the time and costs necessary to decontaminate environments with high contents of heavy metals (KOPTSIK, 2014).

CONCLUSION

For most crops, the toxicity scale of the heavy metal is in the following order (from most to least toxic): Pb \approx Hg > Cu > Cd \approx As > Co \approx Ni \approx Zn > Mn (KOPITKE et al., 2010b). However, forage grasses show specificity in relation to heavy metals, and thus the following toxicity scale can be suggested (from most to least toxic) for this group of plants: Pb \approx Cr > Cd \approx As > Zn \approx Cu \approx Ni > Mn.

The exposure of the forage grasses to the heavy metals resulted in lower biomass production, but this plants can contribute to the process of phytoremediation of contaminated soils as they have higher biomass production than other plant species considered heavy metal hyperaccumulators (e.g., *Noccaea caerulea* and *Arenaria orbiculata*), even with the reported productivity losses; in this regard, *Panicum maximum*, *Avena sativa*, and *Lolium perenne* stood out for being more tolerant to heavy metals than the other evaluated species.

INFORMAL REPORT

1- Heavy metals or trace elements are the commonly used terms for the chemical elements located in the transition group of the periodic table whose density is $\geq 5 \text{ g cm}^{-3}$. However, for the study of heavy metals in plants, density is not considered (APPENROTH, 2010).

2- Lipid peroxidation is the process in which the ROS "attack" the polyunsaturated fatty acids of the phospholipids from the cell membranes, disintegrating them and allowing the entry of these species into the intracellular structures, which leads to the generation of toxic conditions to the plant development (FARMER & MUELLER, 2013).

3- The term phytoremediation refers to the use of plants able to extract dangerous substances from the soil or transform them into safe metabolites to the environment (KOPTSIK, 2014).

4- The term tolerant is utilized to refer to the inherent or acquired ability of a plant to withstand alterations caused by toxic elements without significant impairment to its production (KOPTSIK, 2014).

5- For the ion to be absorbed, it must have contact with the roots by one of the following processes: mass flow and root diffusion or interception (as the root grows, the ions of the soil liquid and solid phases are in contact) (MARSCHNER, 2012).

6- Enzymes that employ metal ions as a cofactor to reduce the activation energy in the breaking of chemical bonds [Adapted from GRATÃO et al. (2005)].

7- Thiol is an organosulfur compound that contains an SH group bound to a carbon atom. Compounds from this group participate in redox reactions and can form strong complexes with metal ions [Adapted from NOCTOR et al. (2011)].

8- Isoforms are distinct forms of a same protein that are transcribed by distinct genes or alternative processes [Adapted from GILL & TUTEJA (2010)].

REFERENCES

- ANDRADE, M.G. et al. Heavy metals in soils of a lead mining and metallurgy area. I - Phytoextraction. *Revista Brasileira de Ciência do Solo*, v.33, p.1879-1888, 2009. Available from: <<http://dx.doi.org/10.1590/S0100-06832009000600037>>. Accessed: Jun. 01, 2015. doi: 10.1590/S0100-06832009000600037.
- APPENROTH, K. What are "heavy metals" in plant sciences? *Acta Physiologiae Plantarum*, v.32, p.615-619, 2010. Available from: <<http://link.springer.com/article/10.1007%2Fs11738-009-0455-4>>. Accessed: Aug. 07, 2015. doi: 10.1007/s11738-009-0455-4.
- ARGENTA, J.A. et al. Anatomical and physiological characteristics of Tanner grass exposed to arsenic. *Amazonian Journal of Agricultural and Environmental Sciences*, v.56, p.13-22, 2013. Available from: <<http://dx.doi.org/10.4322/reca.2013.075>>. Accessed: Jun. 01, 2015. doi: 10.4322/reca.2013.075.
- ASTOLFI, S. et al. Effects of cadmium on the metabolic activity of *Avena sativa* plants grown in soil or hydroponic culture. *Biologia Plantarum*, v.48, p.413-418, 2004. Available from: <<http://link.springer.com/article/10.1023%2FB%3ABIO-0000041095.50979.b0>>. Accessed: Jun. 01, 2015. doi: 10.1023/B:BIOP.0000041095.50979.b0.
- ASTOLFI, S. et al. Cadmium-induced changes in soil biochemical characteristics of oat (*Avena sativa* L.) rhizosphere during early growth stages. *Soil Research*, v.49, p.642-651, 2011. Available

- from: <<http://dx.doi.org/10.1071/SR11158>>. Accessed: Jun. 01, 2015. doi: 10.1071/SR11158.
- BONNET, M. et al. Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll *a* fluorescence and antioxidant enzymes activities of ryegrass (*Lolium perenne* L. cv. 'Apollo'). **Journal of Experimental Botany**, v.51, p.945-953, 2000. Available from: <<http://jxb.oxfordjournals.org/content/51/346/945.abstract>>. Accessed: Jun. 02, 2015. doi: 10.1093/jexbot/51.346.945.
- CHEN, B. et al. Model evaluation of plant metal content and biomass yield for the phytoextraction of heavy metals by switchgrass. **Ecotoxicology and Environmental Safety**, v.80, p.393-400, 2012. Available from: <<http://www.sciencedirect.com/science/article/pii/S0147651312001194>>. Accessed: Jun. 01, 2015. doi: 10.1016/j.ecoenv.2012.04.011.
- DAN, T. et al. Toxicity thresholds for oat (*Avena sativa* L.) grown in Ni-impacted agricultural soils near Port Colborne, Ontario, Canada. **Canadian Journal of Soil Science**, v.88, p.389-398, 2008. Available from: <<http://pubs.aic.ca/doi/abs/10.4141/CJSS07070>>. Accessed: Jun. 01, 2015. doi: 10.4141/CJSS07070.
- FARMER, E.E.; MUELLER, M.J. ROS-mediated lipid peroxidation and RES-activated signaling. **Annual Review of Plant Biology**, v.64, p.429-450, 2013. Available from: <<http://www.annualreviews.org/doi/abs/10.1146/annurev-arplant-050312-120132>>. Accessed: Aug. 07, 2015. doi: 10.1146/annurev-arplant-050312-120132.
- GILABEL, A.P. et al. The role of sulfur in increasing Guinea grass tolerance of copper phytotoxicity. **Water, Air and Soil Pollution**, v.225, p.1806-1816, 2014. Available from: <<http://link.springer.com/article/10.1007/s11270-013-1806-8>>. Accessed: Jun. 01, 2015. doi: 10.1007/s11270-013-1806-8.
- GILL, S.S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v.48, p.909-930, 2010. Available from: <<http://www.sciencedirect.com/science/article/pii/S0981942810001798>>. Accessed: Aug. 05, 2015. doi: 10.1016/j.plaphy.2010.08.016.
- GRATÃO, P.L. et al. Making the life of heavy metal-stressed plants a little easier. **Functional Plant Biology**, v.32, p.481-494, 2005. Available from: <<http://www.publish.csiro.au/?paper=FP05016>>. Accessed: Aug. 05, 2015. doi: 10.1071/FP05016.
- HU, Z. et al. Growth responses of two tall fescue cultivars to Pb stress and their metal accumulation characteristics. **Ecotoxicology**, v.24, p.563-572, 2015. Available from: <<http://link.springer.com/article/10.1007/s10646-014-1404-6>>. Accessed: Aug. 16, 2015. doi: 10.1007/s10646-014-1404-6.
- KOPITTKÉ, P.M. et al. Toxic effects of Pb²⁺ on the growth and mineral nutrition of Signal grass (*Brachiaria decumbens*) and Rhodes grass (*Chloris gayana*). **Plant and Soil**, v.300, p.127-136, 2007. Available from: <<http://link.springer.com/article/10.1007/s11104-007-9395-1>>. Accessed: Jun. 02, 2015. doi: 10.1007/s11104-007-9395-1.
- KOPITTKÉ, P.M. et al. Tolerance of two perennial grasses to toxic levels of Ni²⁺. **Environmental Chemistry**, v.5, p.426-434, 2008. Available from: <<http://www.publish.csiro.au/paper/EN08054>>. Accessed: Aug. 16, 2015. doi: 10.1071/EN08054.
- KOPITTKÉ, P.M. et al. Toxic effects of Cu²⁺ on growth, nutrition, root morphology, and distribution of Cu in roots of Sabi grass. **Science of the Total Environment**, v.407, p.4616-4621, 2009. Available from: <<http://www.sciencedirect.com/science/article/pii/S0048969709003817>>. Accessed: Aug. 18, 2015. doi: 10.1016/j.scitotenv.2009.04.041.
- KOPITTKÉ, P.M. et al. Toxicity of Cd to Signal grass (*Brachiaria decumbens* Stapf.) and Rhodes grass (*Chloris gayana* Kunth.). **Plant and Soil**, v.330, p.515-523, 2010a. Available from: <<http://link.springer.com/article/10.1007/s11104-009-0224-6>>. Accessed: Jun. 02, 2015. doi: 10.1007/s11104-009-0224-6.
- KOPITTKÉ, P.M. et al. Trace metal phytotoxicity in solution culture: a review. **Journal of Experimental Botany**, v.61, p.945-954, 2010b. Available from: <<http://jxb.oxfordjournals.org/content/61/4/945.full>>. Accessed: Aug. 05, 2015. doi: 10.1093/jxb/erp385.
- KOPITTKÉ, P.M. et al. Tolerance of seven perennial grasses to high nickel in sand culture. **Environmental Chemistry**, v.7, p.279-286, 2010c. Available from: <<http://www.publish.csiro.au/paper/EN09100.htm>>. Accessed: Aug. 18, 2015. doi: 10.1071/EN09100.
- KOPTSIK, G.N. Modern approaches to remediation of heavy metal polluted soils: a review. **Eurasian Soil Science**, v.47, p.707-722, 2014. Available from: <<http://link.springer.com/article/10.1134/S1064229314070072>>. Accessed: Aug. 15, 2015. doi: 10.1134/S1064229314070072.
- LAMBRECHTS, T. et al. Comparative analysis of Cd and Zn impacts on root distribution and morphology of *Lolium perenne* and *Trifolium repens*: implications for phytostabilization. **Plant and Soil**, v.376, p.229-244, 2014. Available from: <<http://link.springer.com/article/10.1007/s11104-013-1975-7>>. Accessed: Jun. 01, 2015. doi: 10.1007/s11104-013-1975-7.
- LI, H. et al. Antioxidant enzyme activity and gene expression in response to lead stress in perennial ryegrass. **Journal of the American Society for Horticultural Science**, v.137, p.80-85, 2012. Available from: <<http://journal.ashspublishings.org/content/137/2/80.full>>. Accessed: Jun. 02, 2015.
- LOU, Y. et al. Exogenous glycinebetaine alleviates the detrimental effect of Cd stress on perennial ryegrass. **Ecotoxicology**, v.24, p.1330-1340, 2015. Available from: <<http://link.springer.com/article/10.1007/s10646-015-1508-7>>. Accessed: Aug. 18, 2015. doi: 10.1007/s10646-015-1508-7.
- LUO, H. et al. Antioxidant responses and gene expression in perennial ryegrass (*Lolium perenne* L.) under cadmium stress. **Ecotoxicology**, v.20, p.770-778, 2011. Available from: <<http://link.springer.com/article/10.1007/s10646-011-0628-y>>. Accessed: Jun. 02, 2015. doi: 10.1007/s10646-011-0628-y.
- LUX, A. et al. Root responses to cadmium in rhizosphere: a review. **Journal of Experimental Botany**, v. 62, p. 21-37, 2011. Available from: <<http://jxb.oxfordjournals.org/content/62/1/21.abstract>>. Accessed: Aug. 07, 2015. doi: 10.1093/jxb/erq281.
- MARSCHNER, P. **Mineral nutrition of higher plants**. 3.ed. Londres: Elsevier, 2012. 649p.
- MIRSHEKALI, H. et al. Effect of zinc toxicity on plant productivity, chlorophyll and Zn contents of sorghum (*Sorghum bicolor*) and common lambsquarter (*Chenopodium album*). **International Journal of Agriculture**, v.2, p.247-254, 2012. Available from: <<http://eprints.icrisat.ac.in/4504/>>. Accessed: Aug. 18, 2015.

- MIRZAHOSSEINI, Z. et al. Neotyphodium endophytes may increase tolerance to Ni in tall fescue. **European Journal of Soil Biology**, v.63, p.33-40, 2014. Available from: <<http://www.sciencedirect.com/science/article/pii/S1164556314000594>>. Accessed: Jun. 02, 2015. doi: 10.1016/j.ejsobi.2014.05.004.
- MONNET, F. et al. Photosynthetic activity of *Lolium perenne* as a function of endophyte status and zinc nutrition. **Functional Plant Biology**, v.32, p.131-139, 2005. Available from: <<http://dx.doi.org/10.1071/FP04129>>. Accessed: Jun. 02, 2015. doi: 10.1071/FP04129.
- NIEBOER, E.; RICHARDSON, D.H.S. The replacement of the nondescript term heavy metals by a biologically and chemically significant classification of metal ions. **Environmental Pollution**, v.1, p.3-26, 1980. Available from: <<http://www.sciencedirect.com/science/article/pii/0143148X80900178#>>. Accessed: Aug. 11, 2015. doi: 10.1016/0143-148X(80)90017-8.
- NOCTOR, G. et al. Glutathione. **Arabidopsis Book**, v.9, p.1-42, 2011. Available from: <<http://www.bioone.org/doi/full/10.1199/tab.0142>>. Accessed: Aug. 12, 2015. doi: 10.1199/tab.0142.
- PIRES, M.F. et al. Mechanisms of the internal structure and operation of *Panicum aquaticum* in response to arsenic. **Amazonian Journal of Agricultural and Environmental Sciences**, v.56, p.89-94, 2013. Available from: <<http://dx.doi.org/10.4322/rca.2013.086>>. Accessed: Jun. 01, 2015. doi: 10.4322/rca.2013.086.
- RABÊLO, F.H.S. **Enxofre na atenuação dos efeitos tóxicos do cádmio no capim-tanzânia**. 2014. 103f. Dissertação (Mestrado em Solos e Nutrição de Plantas) - Curso de Pós-graduação em Solos e Nutrição de Plantas, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, SP.
- RIBERA-FONSECA, A. et al. Early induction of Fe-SOD gene expression is involved in tolerance to Mn toxicity in perennial ryegrass. **Plant Physiology and Biochemistry**, v.73, p.77-82, 2013. Available from: <<http://www.sciencedirect.com/science/article/pii/S0981942813003069>>. Accessed: Aug. 18, 2015. doi: 10.1016/j.plaphy.2013.08.012.
- SCHUERGER, A.C. et al. Comparison of two hyperspectral imaging and two laser-induced fluorescence instruments for the detection of zinc stress and chlorophyll concentration in Bahia grass (*Paspalum notatum* Flugge). **Remote Sensing of Environment**, v.84, p.572-588, 2003. Available from: <<http://www.sciencedirect.com/science/article/pii/S0034425702001815>>. Accessed: Jun. 01, 2015. doi: 10.1016/S0034-4257(02)00181-5.
- SCHUTZENDUBEL, A.; POLLE, A. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. **Journal of Experimental Botany**, v.53, p.1351-1365, 2002. Available from: <<http://jxb.oxfordjournals.org/content/53/372/1351.full>>. Accessed: Aug. 11, 2015. doi: 10.1093/jxb/53.372.1351.
- SOLEIMANI, M. et al. Effect of endophytic fungi on cadmium tolerance and bioaccumulation by *Festuca arundinacea* and *Festuca pratensis*. **International Journal of Phytoremediation**, v.12, p.535-549, 2010. Available from: <<http://dx.doi.org/10.1080/15226510903353187>>. Accessed: Jun. 01, 2015. doi: 10.1080/15226510903353187.
- TOLENTINO, T. et al. Especificação do cádmio em *Brachiaria brizantha* e biodisponibilidade dos macro e micronutrientes. **Revista de Ciências Agrárias**, v.37, p.292-298, 2014. Available from: <http://www.scielo.mec.pt/scielo.php?pid=S0871-018X2014000300005&script=sci_arttext>. Accessed: Jun. 02, 2015. doi: S0871-018X2014000300005.
- URAGUCHI, S. et al. Contributions of apoplasmic cadmium accumulation, antioxidative enzymes and induction of phytochelatins in cadmium tolerance of the cadmium-accumulating cultivar of black oat (*Avena strigosa* Schreb.). **Planta**, v.230, p.267-276, 2009. Available from: <<http://link.springer.com/article/10.1007/s00425-009-0939-x>>. Accessed: Jun. 01, 2015. doi: 10.1007/s00425-009-0939-x.
- WEI, L. et al. Copper accumulation and tolerance in *Chrysanthemum coronarium* L. and *Sorghum sudanense* L. **Archives of Environmental Contamination and Toxicology**, v.55, p.238-246, 2008. Available from: <<http://link.springer.com/article/10.1007/s00244-007-9114-1>>. Accessed: Jun. 01, 2015. doi: 10.1007/s00244-007-9114-1.
- WEIHONG, X.U. et al. Effects of insoluble Zn, Cd, and EDTA on the growth, activities of antioxidant enzymes and uptake of Zn and Cd in *Vetiveria zizanioides*. **Journal of Environmental Sciences**, v.21, p.186-192, 2009. Available from: <<http://www.sciencedirect.com/science/article/pii/S1001074208622494#>>. Accessed: Jun. 02, 2015. doi: 10.1016/S1001-0742(08)62249-4.
- WERNER, J.C. et al. Forrageiras. In: RAIJ, B. van et al. **Recomendações de adubação e calagem para o estado de São Paulo**. 2.ed. Campinas: Instituto Agrônomo, Fundação IAC, 1996. Cap.24, p.263-273.
- WYSZKOWSKI, M.; RADZIEMSKA, M. Assessment of tri- and hexavalent chromium phytotoxicity on oats (*Avena sativa* L.) biomass and content of nitrogen compounds. **Water, Air and Soil Pollution**, v.224, p.1619-1633, 2013. Available from: <<http://link.springer.com/article/10.1007/s11270-013-1619-9>>. Accessed: Jun. 01, 2015. doi: 10.1007/s11270-013-1619-9.
- ZALEWSKA, M. Response of perennial ryegrass (*Lolium perenne* L.) to soil contamination with zinc. **Journal of Elementology**, v.17, p.329-343, 2012. Available from: <<http://jsite.uwm.edu.pl/articles/view/197/>>. Accessed: Jun. 01, 2015. doi: 10.5601/jelem.2012.17.2.14.
- ZENG, F. et al. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. **Environmental Pollution**, v.159, p.84-91, 2011. Available from: <<http://www.sciencedirect.com/ez67.periodicos.capes.gov.br/science/article/pii/S0269749110004240>>. Accessed: Jan. 07, 2016. doi: 10.1016/j.envpol.2010.09.019.
- ZHANG, X. et al. Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of an energy crop, king grass (*Pennisetum americanum* x *P. purpureum*). **Biomass and Bioenergy**, v.67, p.179-187, 2014a. Available from: <<http://dx.doi.org/10.1016/j.biombioe.2014.04.030>>. Accessed: Jun. 01, 2015. doi: 10.1016/j.biombioe.2014.04.030.
- ZHANG, X. et al. Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of bana grass and vetiver grass. **Ecotoxicology and Environmental Safety**, v.106, p.102-108, 2014b. Available from: <<http://www.sciencedirect.com/science/article/pii/S0147651314001651>>. Accessed: Jun. 01, 2015. doi: 10.1016/j.ecoenv.2014.04.025.