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Original Research

HMs Induced Changes on Growth, Antioxidant Enzyme's Activity, gas Exchange Parameters and Protein Structures in *Sasa Kongosanensis f. Aureo – Striatus*

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Abstract

Nowadays, the contaminations generated by anthropogenic activities have had damaging effects on the life cycle of plants, particularly on the plants living in the vicinity of urban areas that are more associated with the life of people. Bamboo, as a local plant, is one of the most widely used plants in China. So, a pot experiment was conducted to investigate the effects of three HMs (Cu, Pb, and Zn) in four different concentrations (0 (for control), 500 mg/kg, 1000 mg/kg, and 200 mg/kg), with complete randomized design (CRD) by five replications for each treatment to measure the antioxidant enzymes, lipid per oxidation (LP), soluble protein (SP), gas exchange parameters, and morphological indexes in *Sasa kongosanensis f. aureo – striatus*. The results indicated that the antioxidant responses had an arc-shaped trend with various alterations in which POD and CAT first increase with low concentration of HMs (500 mg/kg) and then decrease with the increase of heavy metal (1000 mg/kg and 2000 mg/kg). Additionally, measuring of MDA content and soluble protein illustrated that MDA content and soluble protein increase with excess of HMs in different levels, and also the excess of HMs significantly decreases the photosynthesis properties. Moreover, the results obtained by morphological indices showed that low concentration of HMs increases both percentage of shoot length and percentage of emerge plants at all three kinds of HMs, but observe a downward trend with excess of heavy metal in 1000 mg/kg and 2000 mg/kg. Overall, in "*Sasa kongosanensis f. aureo – striatus*, results indicated that low concentration of HMs (500 mg/kg) can help the plant growth, while excess of HMs (1000-2000 mg/kg) alleviates the plant growth. On the other hand, Pb revealed the lowest antioxidant

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activity that leads to most membrane damage and eventually showed the lowest plant growth among the cases that HMs were tested, while Zn showed the most increasing plant growth in low concentration.

Keywords: photosynthesis, plant growth, HMs, bamboo, enzyme's activity

Introduction

Today an excess of HMs produced by anthropogenic activities – including disposal of sewage sludge and industry, mining, agricultural operations, and atmosphere contamination – has reached the warning border for underground resources so that the leakage in underground sources and accumulation in agricultural soils has caused many ecological problems [1-2]. Recently, China's industrial development, besides numerous benefits, has created some superabundance environmental challenges such as the emergence of excessive HMs that could raise concerns about the mobility and availability of plants and, consequently, influence their growth and development [3]. There are two kinds of heavy metals in soil, namely essential and nonessential; essential HMs play an important role in many enzymes and other proteins and therefore, plants need them to grow and develop. HM concentration is counted as one important factor in the growth of plants so that excessive amounts of HMs can lead to a reduction in plant growth [4]. In plants, excess HMs cause a high dose of free radicals ($O_2^{\bullet-}$, $OH^{\bullet}$, $OH_2^{\bullet}$) and non-radical hydrogen peroxide (H_2O_2), which eventually leads to the production of reactive oxygen species (ROS) [5-7]. ROS has a destructive reaction on the performance and efficiency of the cell membrane, including proteins, lipids, functions, permeability, and membrane-bound enzymes, and could lead to per oxidation of membrane lipids [5-8]. In fact, plants face some enzymes' mechanisms, including catalase (CAT, EC 1.11.1.6), per oxidases (POD, EC 1.11.1.7), and also a non-enzyme mechanism, including proline when they are exposed to extreme HMs [6-7, 9-10]. POD and CAT play an important role in H_2O_2-scavenging so that the H_2O_2 toxicity can be removed by dimensional reactions of CAT and POD [9-11] .The degradation of H_2O_2 to water and oxygen is carried out by catalase (CAT, EC 1.11.1.6) [5]. However, this may sever the stresses and restrain their enzymatic activity [11].

Bamboo, as a fast-growing plant, covers a wide area of forests in China [3]. *Sasa kongosanensis f. aureo – striatus* or *Pleioblastus kongosanensis f.aureo – striatus*, which is examined in our study, is an ornamental bamboo with clear green leaves and yellow stripes [12] that is known to be a natural antioxidant source [13]. The aims of our study is to investigate the decreasing rate of plant growth when exposed to an excess of HMs and illustrate the effect of bamboo on heavy metals.

Materials and Methods

A greenhouse experiment was conducted to determine toxicity of different concentrations of heavy metals (such as Cu, Pb, and Zn) on the growth and development of one two-year-old ornamental bamboo species called *Sasa kongosanensis f. aureo – striatus*. Different heavy metal concentrations, including 0 mg/kg (control), 500 mg/kg, 1,000 mg/kg, and 2,000 mg/kg, were considered for three heavy metals of Cu, Pb, and Zn that are common in the understudy areas. The experimental design was established under a completely randomized design of CRD with five replicates for each treatment. The R software package was used to analyze the variance of ANOVA using Tukey's test. Significance was determined at $p<0.001$ (*$p<0.05$, **$p<0.01$, and ***$p<0.001$). The tables are expressed as mean values and standard deviation (SD).

Plants were grown under excessive concentrations of heavy metals for a period of 60 days at control conditions. Specific amounts of heavy metals during the 60-day experimental period are summarized in Table 1. The concentrations of elements (500 mg/Kg, 1,000 mg/Kg, and 2,000 mg/Kg) were applied in the form of aqueous solution including ($Cu\ SO_4\ .5H_2O$) _ ($Pb(NO_3)_2$) _ ($Zn(SO_4).\ 7\ H_2O$). The weight of dry soil for pots was 0/710kg = 710 g. After measuring the photosynthesis and morphological indexes, the samples were moved to the physiology laboratory of the Nanjing Forestry University for measuring the protein content and enzyme activity (free from pollution and sterilized).

The sampling leaves were squished in the oven so that all the interior organs were removed. For eliminating the interior tissues of the leaves, nitrogen liquid was used and then the resulting powder was mixed with 2 mg pH 7.8 buffer in a test tube. Then samples were centrifuged at 7,000 rpm for 10 minutes. The estimation of antioxidant activities of peroxidase dismutase (POD, E.C. 1.11.1.7) was carried out according to [14]. The catalase CAT (EC 1.11.1.6) activity was evaluated based on [15]. LP of leaves was measured by estimating Malondialdehyde (MDA) content according to the method of [16] .The soluble protein was used to measure the changes in protein concentration in bamboo species as affected by heavy metal treatments. This protein estimation was done using Coomassie Brapt Blue G25, which is according to the procedure described by [17]. In this experiment gas exchange was measured by using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with light sources consisting

Table 1. Specific amounts of HMs (Cu, Pb, and Zn) in the experiment.

HM concentrations	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Amounts of HMs in pot	355 mg	710 mg	1,420 mg

of blue-red light-emitting diodes (LI-6400-02B). The measurements were conducted at photosynthetic photon flux density (PPFD) of 1,000 µMm−2s−1, leaf temperature of 25ºC, and constant CO_2 of 380±5 µM (CO_2) mol−1 in the sample chamber provided with buffer volume. Morphological indexes included the length of the shoot and the number of emerged plants. To determine the percentage of shoot length, the heights of three to four of the tallest shoots in each pot were measured and their means were computed prior to the experiment. After the application of HM treatments, the mean heights of three to four of the tallest shoots in each pot along with the control ones were measured.

The percentage of shoot length (%) was calculated as length of senesced shoots / length of emerged shoots ×100. The emergence of new bamboo shoots occurred simultaneously during spring. Some of the emerged shoots withered away. So numbers of emerged shoots and senesced shoots were counted, and the percentage of senescent shoots (%) was computed as numbers of senesced shoots / numbers of emerged shoots ×100.

Results and Discussion

Determining Antioxidant Activity

Plants carry a defence mechanism to counter HM stresses, which increase antioxidant enzyme activities such as catalase (CAT, EC 1.11.1.6) or per oxidases (POD, EC 1.11.1.7) [18]. However, the reaction of plants to HMs is generally under the environmental influence of plant genotype [4]. The capacity of a plant antioxidant depends on the production and efficiency of antioxidant defence enzymes in the plant [19]. This means that sometimes the level of antioxidant in the cell membrane is not enough and can reduce the amount of ROS [20]. Our results, compared with control treatment, indicate that the HM concentrations significantly increased the antioxidant enzymes activities (POD and CAT) in *Sasa kongosanensis* (Table 2) to 37%, 31%, and 46% in POD activity, and 106%, 79%, and 127% in CAT activity by Cu, Pb, and Zn, respectively.

Among the reasons for the increase of antioxidant activity in our species, the plant's natural response can be pointed out when it is confronted with heavy-metal stress. This issue has been reported in several studies such as: 1) POD and CAT activities under Cd stress at tomato seedlings [21]; 2) SOD, POD, and CAT activities under Cu stress at *Astragalus neo-mobayenii* [10]; and 3) SOD and POD activities at *Typha angustifolia* under Cr, Pb, and Cd stress [22].

However, the results revealed that the antioxidant activities first increase with low concentrations of heavy metal (500 mg/kg) and then decrease with an excess of HMs at concentrations of 1,000 and 2,000 mg/kg, respectively (Table 2). This increase in antioxidants can be either the plant effort to survive with the accumulation of HMs, or the threshold defence mechanism of plants to avoid oxidative stress with the accumulation of HMs. Therefore, when this phenomenon occurs, that amount of the ROS is more than the capacity of the plant antioxidant enzymes [23]. The excessive amount of superoxide radical could lead to an interaction between the cell compound and the HMs so that it impairs the transmission of signals to the gene used for antioxidants. This interdiction can occur by replacing

Table 2. The effects of HMs (Cu, Pb, and Zn) on the POD, CAT, MDA, and soluble protein of *sasa kongosanensis f. aureo – striatus*. Each data point is a mean value ± SE of five replicates. The capital letters are the demonstration of statistical significance between different concentrations of HMs and the small letters are the demonstration of statistical significance between different HMs in each concentration.

Heavy metal	Treatment	POD	CAT	MDA	SP
	mg/kg	u.g.Fw	u.g.Fw	µmol.g.Fw	mg/g.Fw
Cu	0	0.75± 0.10^{Ba}	0.023 ± 0.004^{Ca}	0.041± 8.10^{Aa}	0.029 ±0.011^{Bb}
	500	1.13± 0.18^{Aa}	0.054± 0.004^{Aa}	0.044± 6.42^{Aa}	0.033 ± 0.008^{Bb}
	1,000	1.02± 0.16^{Aa}	0.047 ± 0.006^{ABa}	0.046± 9.76^{Aa}	0.052± 0.007^{Ab}
	2,000	0.94± 0.08^{ABa}	0.040± 0.006^{Ba}	0.059± 3.87^{Ab}	0.062± 0.007^{Ab}
Pb	0	0.74±0.18 ^{Ba}	0.029± 0.005^{Ca}	0.039± 0.013^{Ca}	0.042± 0.005^{Ca}
	500	1.13±0.19 ^{Aa}	0.061 ±0.007^{Aa}	0.067± 0.015^{Ba}	0.045± 0.008^{Cab}
	1,000	0.95±0.14^{ABa}	0.053± 0.008^{ABa}	0.085± 0.013^{ABa}	0.087± 0.005^{Ba}
	2,000	0.87±0.23^{ABa}	0.044± 0.009 ^{Ba}	0.093± 0.014^{Aa}	0.107 ± 0.009^{Aa}
Zn	0	0.63 ± 0.13^{Ba}	0.020± 0.005^{Ba}	0.043± 0.017^{Aa}	0.041±0.003^{Cab}
	500	0.98 ± 0.22^{Aa}	0.058± 0.007^{Aa}	0.049± 0.012^{Aa}	0.053± 0.005^{BCa}
	1,000	0.91± 0.11^{ABa}	0.049± 0.001^{Aa}	0.051 ±0.011^{Aa}	0.065± 0.014^{ABb}
	2,000	0.89± 0.23^{ABa}	0.032± 0.008^{Ba}	0.051 ± 0.016^{Ab}	0.074± 0.013^{Ab}

and blocking the ions in important groups of cells [24]. This phenomenon has been confirmed and emphasized by many researchers in various plants such as: 1) SOD, POD, and CAT activities under Cr stress in mung beans [25]; 2) antioxidant enzyme activity of *Suaeda heteroptera* under Cu stress [26]; 3) SOD and CAT activity under lead stress in water hyacinths *Eichhornia crassipes* (Mart.) [27]; and 4) CAT activity under Pb stress in *Sesbania grandiflora* [28].

Overall, the present study's antioxidant enzymatic activities have shown an arc-shaped response to different levels of HMs in which antioxidant enzymes first increased at low concentrations of HM and then decreased with an excess of heavy metal. Hence, compared with control treatment, the increased amount at different concentrations of HMs in POD and CAT activities are Zn>Cu>Pb (Table 2).

Determination of Lipid per Oxidation (LP) and Soluble Protein (SP)

Basically, the LP could be used as an indicator of oxidative stress on the plants [9, 29-30]. When the antioxidant enzyme is unable to wipe out the damaging effects of free radicals and ROS in plants completely, the LP causes the oxidation of membrane lipids that can disable the cell structure and membrane of the organelle [9]. The level of the damage is characterized by measuring the MDA content, because increasing MDA controls the oxidative stress rate in plants [31-11]. According to our results, the MDA content significantly increases with increasing HMs compared with control treatments: 22%, 110%, and 15% for copper, lead, and zinc, respectively (Table 2). One of the reasons that this increase can show a lack of enough efficiency of antioxidant enzymes is that due to the increase of ROS amount, the free radicals in the cell membranes will increase, consequently leading to the destruction of cell membranes and increasing the lipid per oxidation in cell membranes of bamboo species. The amount of increase is related to the sensitivity of species to cell damage by ROS [32]. Moreover, the excess of heavy metals could lead to an excess of lipid per oxidation, which may in turn lead to the disruption of either membrane permeability and efficiency or enzyme activities such as H+-ATPase [33]. The effects of copper on S. *alterniflora* showed that the excess of Cu with ameliorate membrane permeability lead to the production of superoxide radicals and oxidative stress in plants, and then lead to the enhancement of lipid per oxidation in cell membrane [34]. This issue has been confirmed in several studies concerning the: 1) effect of lead and Cd in mung bean [35], 2) lead on sedum [36], 3) Cd on in *Linum usitatissimum* L [37], and 4) copper on duckweed, cucumber, and *Silene paradoxa* L [8, 38-39].

Furthermore, in another study [29] indicated that in excess of Cu, there is one direct link between the excess of heavy metal and overproduction of reactive oxygen species (ROS) with an excess of cellular permeability. This link can result in the production of lipid per oxidation in cell membrane, and it can be an impairment in connection and correlation between lipids and proteins in cell membrane. Moreover, ROS with oxidation in protein structure at cell membrane leads to impairment in the amino acid chain and overproduction carbonyl groups [40]. The present work tries to provide strong evidence to show that with an excess of heavy metal, soluble protein content would significantly increase. That amount of the increase is seen to be 68%, 86%, and 57% by copper, lead, and zinc compared with the control treatment (Table 2). It then revealed that damaging effects of HMs lead to the fragmentation of proteins in cell membrane [41].

Gas Exchange Parameters (Photosynthesis Indices)

The proper amount of essential HMs, like Cu, Zn, Co, and Fe, are useful for plant metabolism; however, extreme values of HMs disturb some photosynthesis and metabolism activities in plants. HMs can impact plants directly and indirectly. For example, they indirectly decrease net photosynthesis and transpiration in plants by causing a disturbance in the stomatal structure [42]. HMs directly disturb the structure of thylakoid membranes at chloroplast and photosynthetic proteins, which mainly damage the efficiency photochemistry parameters in the dark-adapted state (fv/fn) and PSII [43], and reduce energy transfers to the reaction centre [44].

Gas exchange parameters in *Sasa kongosanensis f. aureo – striatus* indicated that concentrations of HMs had a negative effect on photosynthesis parameters so that at high concentrations (1,000-2,000 mg/kg) it leads to a significant decrease in the net photosynthetic rate (PN), conductance to H_2O (Cond), intercellular CO_2 concentration (Ci), and transpiration rate (Tr). As indicated in Table 3, all the photosynthetic properties were reduced by rising HM concentrations compared to those within their control. Many researchers have pointed to HMs as an inhibitor in the net photosynthetic rate and other photosynthesis properties [45-46]. The results of one experiment revealed that the impacts of two HMs (Cr and Ag) on Cyanobacterium, *Spirulina platensis* downgraded 17% of the whole chain electron transport activity (WCE), and also indicated that 15 μM of Ag and 100 μM of Cr decreased electron transport and PS2 activities by 55-66% and 61-62 %, respectively. Thus, a high concentration of HMs, such as Cr and Ag, with inhibitory effects on light and energy absorption, make a disturbance in the reaction centre and light harvesting complex (LHc), which consequently leads to a disturbance of chain electron transport and transfer systems at Ps II [47]. In another study, a similar impact has been observed in chloroplasts, BBYs, Thylakoid membranes, and PSII complexes [48]. Generally, HMs lead to the inhibition of net photosynthetic rate (Pn) and intracellular CO_2 concentration [45]. Bazihizina et al. (2015) [38] have shown that "stomatal closure attribution" is the most important reason for reducing photosynthesis in plants that are exposed to copper.

Table 3. The effects of HMs (Cu, Pb, and Zn) on gas exchange parameters (photosynthesis indices) of *sasa kongosanensis f. aureo – striatus*. Each data point is mean value ± SE of five replicates. The capital letters are the demonstration of statistical significance between different concentrations of HMs and the small letters are the demonstration of statistical significance between different HMs in each concentration.

Heavy metal	Treatment	Net photosynthetic rate (Pn)	Conductance to H_2O (Cond)	Intercellular CO_2 concentration (CI)	Transpiration rate (Trimmol)
	mg/kg	μmol CO_2 m^{-2} 1	mol H_2O m^{-2} s^{-1}	μmol CO_2 mol^{-1}	mmol H_2Om^{-2} s^1
Cu	0	64.16±1.30 ^{Aa}	0.10±0.04^{Aa}	26.93±8.10^{Aa}	1.7±0.3^{Aa}
	500	60.22± 2.22^{Aa}	0.09±0.02^{Aa}	23.68±6.42^{Aa}	1.5±0.5^{Aa}
	1,000	55.24± 2.72^{Ba}	0.08±0.03^{ABa}	16.99±9.76^{Aa}	1.4±0.3^{Aa}
	2,000	49.71± 2.52^{Ca}	0.04±0.01^{Ba}	13.84± 3.87^{Aa}	1.3±0.2^{Aa}
Pb	0	64.63±2.13 ^{Aa}	0.15±0.02^{Aa}	25.20±7.44^{Aa}	2.3±0.33^{Aa}
	500	58.25±3.58^{Ba}	0.10±0.03^{ABa}	22.05±3.98^{Aa}	2.2±0.34^{Aa}
	1,000	51.27±3.15^{Ca}	0.09±0.04^{ABa}	20.83±4.18^{ABa}	1.9±0.36^{ABa}
	2,000	41.07±1.87^{Da}	0.06±0.02^{Ba}	18.03±7.13^{Aa}	1.8±0.53^{Aa}
Zn	0	64.26±1.38^{Aa}	0.19±0.03^{Aa}	17.48±5.45^{Aa}	2.1±0.46^{Aa}
	500	62.07±1.92^{ABa}	0.16±0.10^{Aa}	16.48±5.18^{Aa}	1.9±0.23^{ABa}
	1,000	59.50±1.55^{Ba}	0.14±0.06 ^{Aa}	15.57±5.95^{Aa}	1.6±0.35^{ABa}
	2,000	54.64±3.04^{Ca}	0.13±0.04^{Aa}	15.23±3.70^{Aa}	1.4±0.29^{Ba}

A previous study showed the effects of HMs (Cu and Pb) on B. *napus* and implicated the excessive amount of Cu (50 or 100 Mm) in the decline of a number of photosynthetic-related indices, including the rate of photosynthesis (μmol CO_2 m-2 s-1), intercellular CO_2 concentration (μmol CO_2 mol-1), conductance to H_2O (mol H_2O m-2 s-1), and net assimilation, as well as transpiration [30]. It also significantly decreased the transpiration rate, stomatal conductance, net photo synthetic rate, and water use efficiency by Pb [49]. Overall, in the present study photosynthetic properties in three HMs of Cu, Pb, and Zn significantly decreased by approximately 1.18-, 1.29- and 1.14-fold of the control, respectively (Table 3).

Determining Plant Growth (Percentage of Shoot Length and Percentage of Emerge Plants)

One important point in the growth of plants exposed to abiotic stress, such as HM stress, is the ability of plant antioxidant enzyme activities in confronting oxidative stress [50]. The results of analysis on the effects of HMs (Cu, Pb, Zn) on the percentage of shoot length and percentage of emerge plants in *Sasa kongosanensis f* showed that, compared with control treatments, the percentage of plant growth mainly increased with low concentrations (500 mg/kg) of HMs (Cu, Pb, and Zn), and then significantly decreased with an excess of HMs (1,000 and 2,000 mg/kg) (Fig. 1). This reduction was demonstrated in copper, lead, and Zn by 21%, 27%, and 10% of shoot length (Fig. 1a) and 13%, 23%, and 6% of emerged plants (Fig. 1b). A reduction of growth in high concentrations has been reported by lead at *Sesbania grandiflora* and Chinese cabbage cultivars [28-51] by Cu at *halophyte Spartina* (poace) and *Brassicanapus* L. and *Lemna minor* L. [2, 52-53], and by Zn in sugarcane and bean [53-54]. Many factors are involved in inhibiting plant growth when they are exposed to high concentrations of HMs. Zaheer [30] indicated that the effects of copper on the growth rate of B. *napus* significantly decreased with the excess of Cu, in which this decline is attributed to changes in root morphology and impairment of nutrient uptake. Another study by Ali [55] shows that this decrease in wheat plant growth rates is attributed to the mesophilic cell dysfunction because of oxidative stress in leaves. Later, in other studies, it was mentioned that the reason for this matter is decreasing photosynthesis and chlorophyll and increasing MDA [19-56]. The other point that was clearly observed in our study as well is that the excess of heavy metal and inhibition of plant growth can lead to chlorosis, necrosis, and leaf depigmentation [2].

On the other hand, the results indicated that the percentage of plant growth, compared with control treatment, has mainly shown a positive reaction at low concentrations (500 mg/kg) that could lead to a 46%, 22%, and 100% increase in shoot length (Fig. 1a), and 19%, 0%, and 33% increase in emerging plants (Fig. 1b) by copper, lead, and zinc, respectively. This could be counted as an essential element in promoting plant growth. In one study, 5 ppm of Cr^{+6}, Cu^{+2}, Ni^{+2}, and Zn^{+2} increased the shoot size by 13.0%, 59.0%, 35.0%, and 6.6%, respectively [1], and

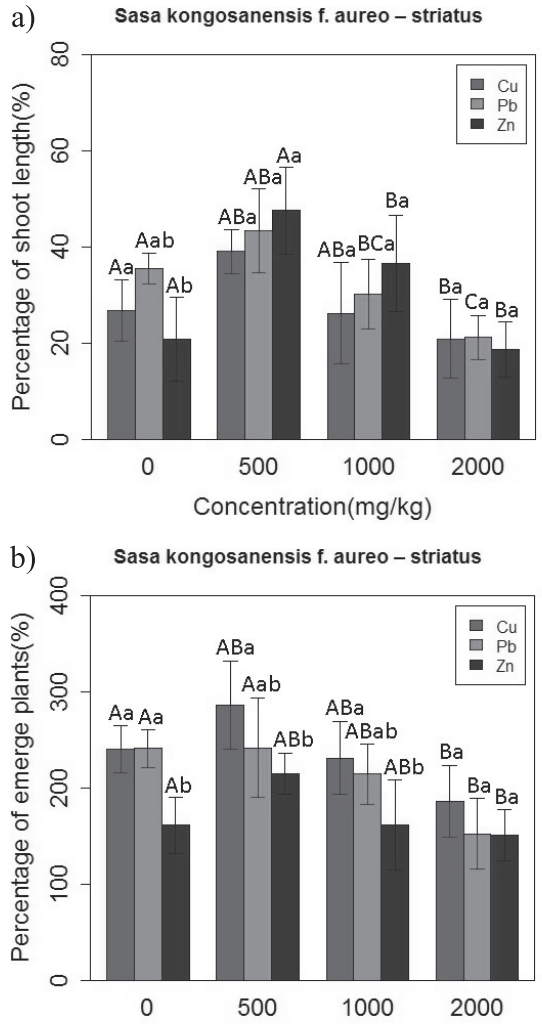

Fig. 1. Effects of HMs on plant growth indices: percentage of shoot length a) and percentage of emerged plants b). The capital letters are the demonstration of statistical significance between different concentrations of HM and the small letters are the demonstration of statistical significance between different HMs in each concentration.

also showed an effect in low concentrations of Cu in *Vicia faba* [31].

Overall, in the present study plant growth (i.e., percentage of shoot length and percentage of emerging plants) is shown to have an arc-shaped response to an excess of HMs so that at low concentrations (500 mg/kg), plant growth indexes increased, and then at high concentrations (1,000 mg/kg and 2,000 mg/kg) plant growth indexes decreased. Among our HMs, Zn was seen to have a high impact of plant growth in low concentrations while Pb was shown to have a high negative effect on plant growth.

Conclusions

Tolerance thresholds to abiotic stress are different in plants and determined by genetics and district environment where the plant grows. The rapid growth of HMs by anthropogenic activities has a negative impact on plant cycles and activates defensive mechanisms, while the logical values of these HMs are mainly used as nutrients in plants. In the present study, the effects of different concentrations of HMs (Cu, Pb, and Zn) on *Sasa kongosanensis f. aureo – striatus* indicated that the low levels of HMs (500 mg/kg) have a positive impact on plant metabolism and can help plant growth, while an excess of HMs (1,000-2,000 mg/kg) mainly decrease growth and put a tolerance threshold in our *Sasa kongosanensis f. aureo – striatus* bamboo species.

Overall, our results showed that the effectiveness of antioxidant mechanisms corresponds to heavy metal stress so that at low concentrations of heavy metals, the enhancement of antioxidant enzyme activity improved the growth and development of plants, while with the excess of heavy metals (1,000 and 2,000), antioxidant enzyme activity decreased. This could enhance lipid production, reduce photosynthetic properties, and, consequently, reduce the plant growth indexes.

Also in our study, Pb was revealed the have the lowest antioxidant activity, which led to most membrane damage and, consequently, showed the lowest plant growth over the three HMs tested in our study, while Zn showed the most increasing growth plant in low concentrations.

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