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A STUDY ON COPPER-TOLERANCE OF GIANT REED (*Arundo donax* L.) ECOTYPES FOR BIOENERGY PURPOSES

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LIST OF ABBREVIATIONS

20SZ	Hungarian ecotype of giant reed
AAS	Atomic Absorption Spectrophotometer
AN	Ammonium nitrate
APEC	The Asia-Pacific Economic Cooperation
BAP	Bioaccumulation percent
BC	Before Christ
BCF	Bioconcentration factor
bl	Billion liters
BL	Blossom ecotype (American) of giant reed
chl *a*	Chlorophyll *a*
chl *b*	Chlorophyll *b*
CSLF	Cellulose solvent-based lignocellulose fractionation
$CuSO_4 \cdot 5H_2O$	Copper (II) sulfate pentahydrate
DM	Dry mass
DTPA	Diethylene triamine pentaacetic acid
DW	Dry weight
EEA	European Environment Agency
EROEI	Energy return on energy invested
ESP	Spanish ecotype of giant reed
FeS	Pyrite
Fv/Fm	Photosynthetic efficiency
G	Guaiacyl
GHG	Greenhouse gases
GWP	Global warming potential
H	*p*-hydroxyphenyl
ha	hectare
kl	kiloliters
LICOR	Infrared gas analyzer
LPO	Lipid peroxidation
m	meter
MBC	Municipal biocompost
MDA	Malondialdehyde
MSSC	Municipal sewage sludge compost
OJIP	Fluorescence induction curve
PbS	Galena
RCBD	Randomized complete block design
ROS	Reactive oxygen species
RR	Removal rate
S	Syringyl
SiO_2	Quartz (sand)
SOM	Soil organic matter
STM	Hungarian ecotype of giant reed
T	Ton
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TF	Transportation factor
WHC	Water holding capacity
ZnS	Sphalerite

1. INTRODUCTION

In recent years much attention has been focused upon improving the grass crops of the world. Prior to the 1955, coal was the main source of power and heat. Next to industrial revolution, world's economy has depended heavily on fossil fuels. Consequently, environmental impacts, especially greenhouse gases (GHG) emissions, are dependent mainly on burning fossil fuels, deforestation and other human activities. Global energy demand has nearly doubled in the last 35 years causing more damage to environment due to fossil fuel use. The EU has set itself the ambitious target to achieve by 2020 a 20% share of renewable energy and biofuel's usage of 10% in transport. Bioenergy represented approximately 7.5% of energy used in the EU in 2010. Currently, bioenergy provides roughly 10% of global supplies and accounts for roughly 80% of the energy derived from renewable sources.

First-generation biofuels "or conventional biofuels are made from sugar, starch, or vegetable oil" can have detrimental consequences for soil carbon sequestration, nitrous oxide emissions, nitrate pollution, biodiversity and human health. Therefore, an alternative is to grow lignocellulosic crops (second-generation biofuels). In recent years much attention has been focused upon improving the grass crops of the world. Lignocellulosic feedstocks can have positive impacts on environment, and could make up a substantial proportion of future energy portfolios. First-generation biofuels may serve as a bridge to second-generation biofuels, which still requires intensive research and development efforts at all levels of the production chain.

At the present, after bioenergy becomes a reality, debate is directed towards suitable lands that can be exploited for biofuel production purposes. As a consequence, for expected growth of the world's population over the decades ahead, agricultural production will have to increase by some 60%. This means more pressure on agricultural lands and water resources whose availability and productivity in agriculture might be under threat from climate change.

Recently, biomass crops such as giant reed were proposed as phytoremediation candidates due to their immense biomass production, fast growing rates and vigorous root systems. Furthermore, these plants are not edible crops and able to grow in a wide range of low nutrient, erodible or metal contaminated soils. In addition, giant reed is a multipurpose plant of promising potential for several non-food uses, especially as an energy crop for the production of bioethanol and energy. Furthermore, obtaining products with economic value such as biomass from plants used in removal pollutants from contaminated soils would be an additional benefit to phytoremediation, which could indeed help sustain its long-term use.

Giant reed displays unique physiological features whereby it readily absorbs and concentrates toxic chemicals from contaminated soil with no appreciable harm to its own growth and development. It is one of the mostly used plants as a trace element bio-accumulator, especially via phytoremediation processes, due to its capacity of absorbing contaminants such as metals that cannot be biodegraded. Giant reed can grow in different environments with spacious ranges of pH, salinity, drought and trace metals without any symptoms of stresses and can easily adapt to different ecological conditions and grow in all types of soils. However, because of its great adaptability to different ecological conditions, giant reed is considered noxious invasive weeds in riparian habitats throughout the world.

Cu is an essential micronutrient that is found in the upper layer of soils. Naturally, the total Cu contents in soils range between 2-109 mg kg^{-1} and world-soil average is 38.9 mg kg^{-1}. Cu is a rather immobile element in soils and shows relatively little variation in total contents of soil profiles. The Cu content is strongly associated with soil texture, where light sandy soils contain less Cu than loamy soils. Soils usually restrain Cu in many forms depending upon soil pH.

Cu, an essential metal in plants, functions as a catalyst in photosynthesis and respiration. It is a constituent of several vital enzymes involved in building and converting amino acids to proteins. Cu is important in carbohydrate and nitrate metabolism. It is essential to the formation of lignin in plant cell walls which contributes to the structural strength of the cells and the plant. It also affects water permeability, reproduction, and disease resistance. In soil, Cu is the most immobile micronutrient. Therefore, Cu uptake is strongly correlated with root systems, where vigorous root systems take up more Cu. Initially, Cu is equally distributed in the plant but is relatively immobile. Moreover, for better plant growth, adequate Cu supply is needed. The appropriate Cu concentration in plants ranges between 8-20 mg kg^{-1}. Otherwise, poor growth, delayed flowering, and plant sterility are the foremost symptoms of insufficient Cu supply. Moreover, Cu deficiency in plant growth may appear as wilting with leaf tips turning a bluish-green color. In cereals plants, the tips may become brown and appear to mimic frost damage. Additionally, young tissues show chlorosis, distortion, and necrosis. The death of the growing points often leads to excessive tillering in cereal crops and excessive branching in dicots. Under toxic Cu levels, plants appear stunted and are usually bluish in color, and eventually turn yellow or brown. Also, seed germination, plant vigor and iron intake are reduced. Furthermore, Cu-induced chlorosis and root malformation are the most common characteristic symptoms of this toxicity.

The objectives of this study were:

- Assess the possible use of Cu-contaminated soil for purposes of bioenergy production during the phytoremediation process.
- Study the growth dynamics of giant reed ecotypes under elevated Cu concentrations in water bodies as well as in Cu-contaminated soil-like growth medium (sand).
- Determine the threshold of Cu on growth of biomass candidate giant reed.
- Calculate the bioconcentration and transportation factors, bioaccumulation percent, and removal rate of giant reed ecotypes for Cu.
- Evaluate the Cu-tolerance capacity of different giant reed ecotypes originated from epigenetic modifications.
- Measuring the efficiency of giant reed ecotypes to clean Cu-contaminated water bodies.

2. LITERATURE

2.1. Bioenergy: past, present, and future

Prior to the 1955, coal was the main source of power and heat. Next to industrial revolution, world's economy has depended heavily on fossil fuels. Consequently, environmental impacts, especially greenhouse gases (GHG) emissions, are dependent mainly on burning fossil fuels, deforestation and other human activities (IEA, 2009). By 2020 the world's population is expected to reach approximately 8 billion. When we consider both the expected increase of worldwide population and the increasing energy prices that are a consequence of ever greater levels of industrialization, it becomes clear that in the decades ahead it will be a significant challenge to provide energy with competitive prices for the world's people, a problem that will increase with time (Popp et al., 2014). As a consequence for expected growth of the world's population over the decades ahead, agricultural production will have to increase by some 60% (FAO, 2011). This means more pressure on agricultural lands and water resources whose availability and productivity in agriculture might be under threat from climate change. Moreover, the incredible prices, limited supply and environmental risks of fossil fuels have made the production of biofuels an urgent and to reach expected volumes over the last decade (Popp et al., 2014).

Global energy demand has nearly doubled in the last 35 years causing more damage to environment due to fossil fuel use (IEA, 2009). Where, during last decade from 2001 to 2011 the total energy consumption throughout the world increased from 77,245 to 88,034 thousand barrels per day. Almost one third of the total world energy consumption is accounted for Asia Pacific (BP, 2012). This inevitability increase in energy consumption leads to fast depleting of fossil fuel reserves around the world. Moreover, many detrimental effects of burning fossil fuels on environment have been recorded specially during last decades such as increasing emissions of GHG and its effect on global warming and climate change. Therefore, energy security and climate change mitigation are two main drivers that have pushed renewable energy production to the top of the global agenda (Karp and Shield, 2008).

In the EU the transportation sector, which is currently entirely relying on fossil fuels and will continue to experience high growth in the decades ahead, is responsible for around 21% of anthropogenic GHG emissions. In order to restrain a fast growing GHG emission profile, biofuels are considered a key solution together with fuel saving vehicle technologies. Continued dependence on fossil fuels will make it very difficult to mitigate emissions of GHG that contribute to global warming and climate change (Crutzen et al., 2008). The detrimental effects of climate change and the threat of diminishing fossil fuel reserves are forcing society to search for renewable sources of energy. Energy can be derived from the biomass of plant material by co-fire combustion with coal or on its own for the production of electricity. Renewable energy carriers can also be created by converting the plant biomass into ethanol, a gasoline substitute. Of course, there is no time to loss, to deliver energy for this growing population; the world needs to start looking for an alternative source of energy, and to do so in a sustainable and environmentally friendly approach to avoid more damage to environment (Popp et al., 2014).

Energy derived from plants, especially biomass species, is an alternative fuel that is expected to play an indispensable role in future world energy portfolio. Therefore, much attention has been paid to energy plants in recent years. Biomass plants will have an important role in the implementation of sustainable bioeconomy. Development of sustainable bioenergy will not only contribute to the energy security, but also will have additional economic and environmental benefits such as GHG emissions mitigation and increase soil carbon sequestration (Wang, 2005). Biomass energy is the most abundant and versatile type of renewable energy in the world (Lin et al., 2007). Developing the knowledge and skills is required for obtaining increased quantities of biomass production that is suitable for conversion to biofuels and to other renewable resources, at economically competitive prices, all within an environmentally and economically sustainable agricultural system that is an essential part of a future bioeconomy (Popp et al., 2014).

In order to ensure the continuity and sustainability of renewable energy production, many countries have made it embodied legislation (e.g. the EU, Directive 2009/28/EU EU, 2009) to decrease dependence on fossil fuels and diminish greenhouse gas emissions (Goldemberg, 2007). The extraordinary political support has quickly pushed the first-generation biofuels "or conventional biofuels are made from sugar, starch, or vegetable oil" to gain public interesting all over the world (Boucher, 2012).

However, this attention has fall down soon because many detrimental consequences of first-generation biofuels quickly surfaced (Fargione et al., 2008). These concerns included food security and energy production debating (Boucher, 2012; Rathmann et al., 2010), detrimental consequences for soil carbon sequestration, nitrous oxide emissions (Crutzen et al., 2008), nitrate pollution (Donner and Kucharik, 2008), biodiversity (Landis et al., 2008) and human health (Hill et al., 2009).

Therefore, interest turned into second generation biofuels that include using of food wastes, agricultural residues, woody plants, and grasses. Nowadays, lignocellulosic crops are the main source of second generation biofuels, where growing biomass plants, which are non-food plants, is the best alternative to avoid present food versus energy argument (Lovett et al., 2014). Recently, much attention has been paid to develope the grass crops of the world. In addition, lignocellulosic feedstocks can have positive impacts on environment (Robertson et al., 2011), and could make up a substantial proportion of future energy portfolios (Perlack et al., 2011). First-generation biofuels may serve as a bridge to second-generation biofuels (Fischer-Boel, 2008), which still requires intensive research and development efforts at all levels of the production chain.

2.1.1. Importance of bioenergy plants

Energy production from second-generation biomass candidates is growing fast in both developed and developing countries to meet mandated increase of biofules that have been legislated (USC, 2007). This requires novel feedstocks - *particularly perennial grasses* - which are fast growing, high yield, and can grow on marginal land (not suited for food crops). Bioenergy plants include several types: (1) energy plants for bio-ethanol, mainly including plants with high content of saccharide, starch and fiber; (2) energy plants for biodiesel, especially non-food oil plants, etc. China has 1554 species of oil plants, including 154 species with oil content greater than 40% in seeds, and 30 species of shrubs or arbor plants with rich bio-fuel components (Shao and Chu, 2008).

Perennial grasses, which once established can be harvested and re-grow annually for many years, have a number of other beneficial characteristics which suit them as biomass crops. These include high resource use efficiency, high productivity, good environmental qualities and a wide range of end uses. Environmental benefits include high rates of soil carbon sequestration, diminish greenhouse gas emissions from fossil fuels and enhanced biodiversity and soil stabilization. Economical benifits include reducing fossil fuel prices and minimize or avoid any negative impact on the global food supply where it is able to grow on marginal areas of land (Jingying et al., 2014).

Hill et al. (2006) reported that burning liquid biofules reduces GHG emissions by 12 – 41% compared to fossil fuels. Combustion of bioethanol decreased GHG by 12%, while using biodiesel recorded 41% decrease in GHG emissions. Moreover, approximately 40% less of GHG emissions were determined by Adler et al. (2007) when ethanol and biodiesel extracted from corn were used as alternative to traditional fossil fuel (Jingying et al., 2014). This reduction of GHG emissions was enhanced when reed canary grass was used as origin of bioethanol and biodiesel recording 85% reduction in GHG emissions. However, using ethanol and biodiesel extracted from hybrid switch grass and poplar decreased GHG emissions by 115% (Adler et al., 2007).

In the UK, Stephenson et al. (Jingying et al., 2014) measured the global warming potential (GWP, in kg CO_2-equivalent) for biodiesel. He reported that biodiesel saved 26 – 32% of GWP when large and small scale production of biodiesel, respectively, were applied compared to ultralow sulfur diesel (Stephenson et al., 2008). The current world biomass demand for mandated energy is estimated to be 53 quintillion joules (REN, 2012). Generally, world energy demand is expected to grow by 35% even with enhancing energy use effeciency. In developing countries energy demand is expected to grow by 65% by 2040 compared to 2010 as direct consequence for developing economies and growing populations. According to the new public energy outlook, 75% of the world's population will reside in Asia Pacific and Africa by 2040. India will have the largest population after 2030 (ExxonMobil, 2013). A wide range of indicators suggest that dramatic developments are taking place in Asian energy markets (Mochizuki et al., 2004) and large-scale bioenergy development is extremely urgent. Schröder et al. (2008) considered bioenergy development as an effective way to save the world from an energy crisis.

2.1.2. Bioenergy production throughout the world

Recently, bioenergy has proven efficiency and possibility to partially offset relying on dwindling fossil fuels and controls the inflated prices of traditional fuels. Consequently, the EU and China have set an ambitious target to achieve by 2020 a 20% share of renewable energy in the total energy usage (EC, 2008; Tang et al., 2010). The EU plans to share 10% of fuels used in transport from biofuels. In 2010 bioenergy represented 7.5% of total energy usage in the EU, and European Environment Agency (EEA) wishes to raise it to almost 10% by 2020. Currently, bioenergy provides roughly 10% of global supplies and accounts for roughly 80% of the energy derived from renewable sources (IEA, 2009). China produced 1.82 million tones of biofuels in 2008 including 1.46 million tons of bioethanol and 0.36 million tons of biodiesel (Tang et al., 2010). By 2020 China expects to produce 12 million tons of biofuels from non-food crops, including 10 million tons of bio-ethanol and 2 million tons of biodiesel (Wang, 2005). The US plans to achieve production of 79 billion liters bioethanol by 2022. To meet the 2022 US biofuel target mandate, Qianlai et al. (2013) reported that many actions must be taken such as intensification and expanding of maize cultivation, establishment of biomass crops such switchgrass and Miscanthus.

Depending on data released from model simulations to evaluate bioenthanol production from maize, Miscanthus, and switchgrass. Qianlai et al. (2013) estimated that 3.0-5.4 thousand liters of bioethanol can be extracted from maize plants per hectare, while extracted bioethanol from Miscanthus was more than twice compared to maize. But switchgrass recorded the lowest production of bioethanol relative to maize and Miscanthus. To achieve the target of biofuel production using maize, about 26.5 million hectares of land and around 90 km^3 of water are needed. But substitution of maize by Miscanthus would save 50% of land and 30% of the water. Developing biofuel conversion technology from Miscanthus would save more land and water, where 9 million hectares of land and 45 km^3 of water would be enough to meet the mandated target of biofuel production. Therefore, Miscanthus could be recommended as alternative biomass crop to maize. Since 2009, annual bioethanol production in the US exceeded 40 billion liters (bl) and in 2011 reached 52.6 billion liters (RFA, 2012). By 2022, the US will produce around 136 billion liters of bioethanol fromrenewable sources including 79 billion liters from biomass candidates (USA, 2007). However, there are two major limitations for cellulosic feedstocks production. Firstly, the available land that can employ for cultivation of biomass plants, anyhow, marginal lands can play a key role to solve this problem. Secondly, the productivity of cellulosic crops grown on marginal lands that already are poor soils will be expected to be low especially without adding fertilizers.

2.1.3. Why marginal land?

Food security and efficiency of agricultural production mainly determine land use for food and feed. In addition to competition with food and feed, biomass production for energy purposes also will add more pressure on land use (Popp et al., 2014). At the present, after bioenergy becomes a reality, debate is directed towards suitable lands that can be exploited for biofuel production purposes. Converting fertile lands to biofuels production faces huge arguments. Where, directing cultivated lands towards biomass production would have possible consequences on environment; therefore, a thorough analysis is needed in order to maximize the biomass production (Fischer et al., 2010). Therefore, in order to circumvent the recent food versus biofuel debate, it would be practical to use marginal lands that can serve as prime real estate for meeting the nation's alternative energy production goals.

Although, nowadays the main portion of biomass energy used for heat and electricity is derived from forests, the dedicated agricultural land to the production of biofuels is growing (Fischer et al., 2010). In Europe land use efficiency of the presently dominating first-generation feedstocks is low compared to estimated potentials for second-generation feedstocks (Fischer et al., 2008). Although much of the land need could be satisfied by growing high-productivity feedstocks on fertile land now used for grain production (Somerville et al., 2010), this strategy fails to consider the long-term need for fertile land to meet future food demands and the need to abate the indirect GHG impacts created when land now in grain ethanol production was originally diverted to biofuel use (Searchinger et al., 2008). Because fertile lands, which are suitable for food production, are fairly limited, bioenergy production may depend on marginal land (Jingying et al., 2014). Therefore, growing cellulosic crops on marginal lands could be a substantial alternative in order to circumvent the recent food versus biofuel debate. Furthermore, obtaining products with economic value from plants grown on low quality lands, not suitable for food crops, would be a great benefit and help sustain its long-term use (Bañuelos, 2006).

However, development of growing bioenergy plants on marginal land should not affect ecology of regional environment, so natural forests, pasturing areas, and other reserves have to stay away from exploiting it for bioenergy purposes. The availability of land to grow energy plants is the key factor for the development of bioenergy production (Dafang et al., 2011).

2.1.4. Definition of marginal land

Although, the term "marginal land" is used in research reports from long time ago, it has not been clearly defined. Recently, marginal land introduced itself as possible alternative for bioenergy production in order to avoid food versus bioenergy debate. However, many studies have introduced definition for marginal land in a subjective sense for non-optimal land for crop production without sufficient reasons (Brian et al., 2014).

Here we display some literatures that defined marginal land. Gelfand et al. (2013) said marginal lands are frequently defined as "those poorly suited to field crops because of low crop productivity due to inherent edaphic or climatic limitations or because they are located in areas that are vulnerable to erosion or other environmental risks when cultivated". In the same way, Dafang et al. (2011) gave definition for marginal land as "land that has relatively poor natural condition but is able to grow energy plants, or land that currently is not used for agricultural production but can grow certain plants". FAO, (1997) cited that "land that is unsuitable for traditional row crops, generally referred to as "marginal" land, may be appropriate for grazing or other uses, including biomass crop production". "Marginal lands are often described as degraded lands that are unfit for food production and/or of some ambiguously poor quality and are often termed unproductive" (Shortall, 2013). Milbrandt and Overend, (2009); Blake et al. (2012) reported that "unproductive soils are characterized by unfavorable chemical and/or physical properties that limit plant growth and yield, including low water and nutrient storage capacity, high salinity, toxic elements, and poor texture".

However, contaminated soils with potential toxic metals such as copper (Cu) are considered marginal lands because these soils will produce metal-laden plants causing a serious threat to human health and food chain. Since metals are non-biodegradable contaminants, it is obligatory to remove these metal ions from soils in order to restore these soils again for food crop production. Many technologies were proposed to decontaminate the metal-contaminated soils, but still phytoremediation, the "plant-based green technology" is the most appropriate, cost-effective and environmentally-friendly technology (Elhawat et al., 2014).

However, from all of the mentioned above we can give a short and simple definition for marginal land as *land that does not fulfill the requirements for safe, sustained, and economical agricultural crop production*.

Marginal lands could meet the requirements of second-generation biofuels such grasses and perennial plants which possess vigorous root systems that already are adapted to low nutrient, erodible or droughty soils. Using such lands for biofuels production will have many environmental benefits because cellulosic crops are able to remove significant amounts of CO_2 from atmosphere during its growth season compared to pre-existing vegetation avoiding local carbon debt (Searchinger et al., 2008). Consequently, substantial reduction of GHG emissions will be recorded without any risks of indirect carbon costs due to displace food and feed production (Searchinger et al., 2008). However, the availability of marginal lands for biomass production and the resulting implications for GHG emissions is still uncertain (Fargione et al., 2008). Establishment of biomass mega-farms on marginal lands will restore the ecosystems of these soils and improve its productivity thus it will contribute even more to bioenergy and GHG mitigation goals in addition to providing greater conservation benefits (Gelfand et al., 2013). Biofuels are widely considered to be a major renewable energy source that has the advantage of mitigating global warming (Tilman et al., 2009, Fargione et al., 2010, Beringer et al., 2011).

2.1.5. Total area of marginal lands around the world

In Europe, the total available area for cellulosic production would be almost 44-53 million hectares of fertile agricultural land by 2030. Additionally, 19 million hectares of pasture land are expected to be converted into biomass production. However, most of these lands are located in Eastern Europe, where directing the cultivated lands towards biomass production is possible (Fischer et al., 2010). Lu et al.

(2012) reported that around 9.48 million hectares of lands are available as marginal land in Canada for bioenergy production. He added that if this land directed towards biofuel production it would supply 33 million tons (using switch grass) or 380 million tons (using hybrid poplar). In Japan, Hattori and Morita (2010) investigated the availability of lands for bioenergy production and evaluated different energy plants. The results showed that rice can be grown as an energy crop in unused low-land paddy fields.

Development of biofuel production in China is maily restricted by limited land sources; therefore exploitation of marginal land for bioenergy purposes is the solution. (Dafang et al., 2011) mentioned that total area of marginal land suitable for growing bioenergy plants on a large scale was about 43.75 million hectares. They also estimated that if 10% of this marginal land was fully utilized for growing the energy plants, the production of bio-fuel would be 13.39 million tons. The available areas in Asia with marginal land suitable for *Cassava*, *Pardanthus chinensis*, and *Jatropha curcas* L. were established to be 1.12 million, 2.41 million, and 0.237 million km^2, respectively, (Jingying et al., 2014). According to The Asia-Pacific Economic Cooperation (APEC), the estimated marginal lands make up approximately 400 million hectares across Asia, the Pacific Islands, Australia, and North America (Milbrandt and Overend, 2009). Other estimates put the global marginal land area anywhere from 1100 (Cai et al., 2011) to 6650 million hectares (FAO, 1997), depending on the parameters used to describe marginal (e.g, “nonfavored agricultural land,” “abandoned or degraded cropland,” or arid, forested, grassland, shrubland, or savanna habitats). The potential area available in the USA for cellulosic and perennial biomass crops ranges from 43 to 123 million hectares (Cai et al., 2011; Campbell et al., 2008). The differences in these estimates reflect the inconsistencies in the usage of the term “marginal land,” despite its common use in the bioenergy industry and literature (Cai et al., 2011; Shortall, 2013; Richards et al., 2014).

2.1.6. Climate change and marginal land

According to Lauren et al. (2015) climate change will exacerbate the issue of land marginalization and degradation (Hatfield, 2014). It has been predicted that negative impacts of climate change will increase over the next 25 years, with longer growing seasons (frost-free periods), increasing extreme precipitation events (both flooding and drought), fewer chilling hours, and a greater number of hot nights projected for most growing regions of the country (Hatfield, 2014). Because these changes are projected to occur to a greater extent in certain regions of the country (Hatfield, 2014), crop production — potentially including biomass crops on marginal lands — may shift to novel regions where different stressors are present or growers may shift to different production systems. For example, to escape the predicted hot nights in the southeast, growers may shift to more northern regions where precipitation may be less frequent (Hatfield, 2014). These changes will — and already do — directly affect the physiology and reproductive development of many crop plants, including first generation biomass crops (Schlenker and Roberts, 2009 ; Lobell et al., 2011). Therefore, it will be important to select the most tolerant crops suitable for future climate scenarios (Oliver et al., 2009).

2.2. Giant reed

2.2.1. Morphology of giant reed

Giant reed (*Arundo donax* L.) is a vigorous erect perennial grass species (Poaceae family), native to the freshwater regions of Eastern Asia, but nowadays considered as a sub-cosmopolitan species given its worldwide distribution (Bell, 1997; Alshaal et al., 2013). It is a hydrophyte, growing along lakes, streams, drains and other wet sites. Under ideal growth circumstances giant reed can reach up to 14 m height (Bacher et al., 2001) and is among the fastest-growing terrestrial plants. It can produce more than 50 t ha^{-1} aboveground dry biomass with average 20-35 t ha^{-1} (Tucker, 1990; Sharma et al., 1998; Günes and Saygin, 1996). Individual tough and hollow stems, 3–5 cm in thickness, have a cane-like appearance similar to bamboo with alternate leaves, 30–60 cm long and 2–6 cm broad, tapered tips and hairy tuft, at the base. Stems produced during the first growing season are unbranched and photosynthetic (Bell, 1997).

Giant reed possesses vigorous root system of rhizomes that spreads widely under the soil surface from 5 to 30 cm in depth. The rhizomes (3–8 cm wide and 10–25 cm in length) produce fibrous roots that can reach 5 m in depth under perfect growth conditions (Bell, 1997; Frandsen, 1997; Sharma et al., 1998). The giant reed stem is a hollow, segmented culm that measures from 1 to 4 cm in diameter and is able to branch. The culms’ walls range from 2 to 7 mm in thickness, and the internodes can reach 30 cm in length. This stem structure is able to support the erect position of such a tall plant, as its mechanical stability is not dependent on turgor pressure (Spatz et al., 1997). Several stems grow from the rhizome buds during all the vegetative season, forming dense clumps. Under optimal conditions stems can grow 10 cm per day, placing it among the fastest growing plants (Perdue, 1958; Bell, 1997). The culms carry alternate leaves that originate from the nodes and are very hard and brittle with a smooth glossy green surface that turns golden yellow at the end of the growing season (Perdue, 1958; Frandsen, 1997; Spatz et al., 1997). Giant reed spreads from horizontal rhizomes below the soil and forms dense stands on disturbed sites, sand dunes, wetlands, and riparian habitats. As a consequence of its high and fast biological productivity, giant reed is widely cultivated to yield non-food crop that can meet requirements for energy, paper pulp production, bio-fuels and construction of build materials, but it has other different uses such as music tools with stem, medicine with roots and soil erosion control through re-vegetation (Alshaal et al., 2015).

2.2.2. Growth conditions of giant reed

Giant reed showed capability to survive under extreme growth conditions, where it can grow on different types of soil with wide ranges of pH, electrical conductivity, and nutrient levels. It can grow in heavy clays to loose sands and gravelly soils. After the first year of growth, giant reed showed more ability to grow on lands with high water and salinity contents; also it can still alive on marshes (Perdue, 1995). Under Mediterranean climate conditions, giant reed can grow year-round but optimal growth occurs between February and October. It grows well when the water table is close or at the soil surface but will be retarded if there is lack of moisture in the first year of growth. Droughts have little effect on the established stands that are in the second or third year of growth. Giant reed can survive extended droughts because of the drought-resistant rhizomes and roots that can reach water supplies. It also can survive very low temperatures in the dormant winter season but is subjected to possible damage with frost events after the start of the spring growth. Its vigor makes giant reed an effective potential competitor for other plant species. Once established, giant reed tends to cover large areas with dense clumps, compromising the presence of native vegetation not able to compete (Bell, 1997; Coffman et al., 2010).

2.2.3. Biomass production of giant reed

From the energy point of view, giant reed can be used to produce energy by direct combustion or to produce second generation bio-fuels, such as bio-ethanol, generated by alcoholic fermentation of lignocellulosic biomass pre-treated to facilitate sugar release (Scordia et al., 2012). Of course, the pre-treatment processes used to increase carbohydrate degradability (e.g. steam explosion, acid and heat treatment) consume energy. Giant reed is one of the tallest herbaceous plants, its lignin content is similar to that of other grass species, with an average value equal to 21%, while it equals to 19% in *switchgrass*, 20% in sugarcane, and 23% in *miscanthus.* The latter values are however still higher than in maize, with lignin content close to 11%. Lignins of *A. donax* are composed of guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units with an S/G ratio of 1.13 - 1.32, which is a little lower value than in maize (Méchin et al., 2000). In fact, Jeon et al. (2010) showed that little differences were noticed, for example, between sorghum straw and giant reed capabilities to produce ethanol after acid pre-treatment and enzyme hydrolysis using *Zymomonas mobilis*. In another work, giant reed, elephantgrass (*Pennisetum purpureum*), Miscanthus and Sugarcane were compared for their capacity to produce bio-ethanol using cellulose solvent-based lignocellulose fractionation (CSLF) pre-treatment and enzymatic (cellulase) hydrolysis (Ge et al., 2011). The results obtained showed no significant differences among these energy crops, consequently indicating that one of the most important parameters to be considered to optimize the EROEI value (Energy return on energy invested) is the biomass production per hectare. Another promising way to use these energy crops is through the direct anaerobic digestion of chopped biomass to produce biogas without any pre-treatment. However, for both bio-ethanol and biogas production, a higher susceptibility of the biomass to pre-treatments will allow the use of more environmentally friendly processes, lowering pollution and energy costs.

2.2.4. Phytoremediation capacity of giant reed

Phytoremediation is the exploitation of plants to successfully treat pollutants in soil, water, and air, encompassing a number of methods for the degradation (phyto- and rhizodegradation), removal (phytoextraction, rhizofiltration, and phytovolatilization), or immobilization (hydraulic control and phytostabilization) of contaminants. It is a resurgent field of ecological research because of the advantages of its being environmentally friendly, safe, and cost-effective (Mirza et al., 2010; Malik, 2007). Hyperaccumulation as a tool for cleaning up metal contaminated environments has been widely suggested (Chaney, 1983). However, hyperaccumulator plants are often linked with slow growth rate and low biomass production, so that net removal of metals via phytoextraction is quite limited. Therefore, a rapidly growing nonaccumulator could be engineered to achieve some of the properties of hyperaccumulators (Shu et al., 2002).

Recently, biomass crops such as giant reed were proposed as phytoremediation candidates due to their immense biomass production, fast growing rates and vigorous root systems (Alshaal et al., 2014). Furthermore, these plants are not edible crops and able to grow in a wide range of low nutrient, erodible or metal contaminated soils. In addition, giant reed is a multipurpose plant of promising potential for several non-food uses, especially as an energy crop for the production of bioethanol and energy (Nassi et al., 2010). Furthermore, obtaining products with economic value such as biomass from plants used in removal pollutants from contaminated soils would be an additional benefit to phytoremediation, which could indeed help sustain its long-term use (Bañuelos, 2006).

Giant reed displays unique physiological features whereby it readily absorbs and concentrates toxic chemicals from contaminated soil with no appreciable harm to its own growth and development (Perdue, 1958; Alshaal et al., 2013; Nsanganwimana et al., 2014; Simon et al., 2009). It is one of the mostly used plants as a trace element bio-accumulator, especially via phytoremediation processes, due to its capacity of absorbing contaminants such as metals that cannot be biodegraded. Giant reed can grow in different environments with spacious ranges of pH, salinity, drought and trace metals without any symptoms of stresses and can easily adapt to different ecological conditions and grow in all types of soils. However, because of its great adaptability to different ecological conditions, giant reed is considered noxious invasive weeds in riparian habitats throughout the world (Coffman et al., 2010). The capacity of giant reed as phytoremediation plant exists in many literature (Shabana et al., 2012; Bonanno, 2012; Miao et al., 2012; Sagehashi et al., 2011; Mirza et al., 2011). Howeve, there is limited data on the capacity of giant reed to recover Cu-contaminated environments.

2.3. Copper

2.3.1. Discovery and history of copper

Copper (Cu) is one of the first metals used by humans along with gold, with a history of at least 10,000 years. Cu derives from the Latin cuprum, derived from the word cyprium, because Cyprus was the main source of Egyptian and Roman Cu (Schroeder et al., 1966). It is well documented that Cu has been part of the human civilizations since ancient times up to present days. It estimates that Cu was discovered at 9000 BC in the Middle East; a Cu-pendant was found in northern Iraq that dates to 8700 BC. Cu fabricated in Mesopotamia was quickly introduced to the the Egyptian Empire where its use flourished thousands of years. These peoples used Cu for the fabrication of different jewels, ornaments, but also in the fields of armament and tools. They soon realized that pure Cu, because of its softness, is not suitable for shaping tools used in agriculture. Later, in the era of the Romans other metals, especially iron and bronze as the main metals in weaponry, gained an importance. In that time Cu was first used also for architectural intentions, what can be witnessed on the roof sheathing of the Pantheon (Rusjan, 2012).

2.3.2. Cu concentrations in soils

Cu is a redox-active transition metal that is found in the upper layer of soils. Naturally, the total Cu contents in soils range between 2-109 mg kg^{-1} and world-soil average is 38.9 mg kg^{-1}. Cu is a rather immobile element in soils and shows relatively little variation in total contents of soil profiles. The Cu content is strongly associated with soil texture, where light sandy soils contain less Cu than loamy soils (Kabata-Pendias, 2011). The concentration of Cu in a soil can be increased by applying fertilizers or organic wastes containing Cu. Piggery wastes and digested urban sewage may contain relatively large concentrations of Cu. The soils in horticultural areas may also contain elevated concentrations of Cu as a result of using Cu compounds to control plant pests and diseases (Cornforth et al., 2003). The regularity in large-scale Cu occurrence in soils indicates that two main factors, parent material and soil formation processes, govern the initial Cu status of soils. Also, the clay fraction contributes significantly to the Cu content of soils. Other soil properties, such as Fe and Mn oxides, and base saturation, explain about 15–25% of all impact factors (Kabata-Pendias, 2011).

Concentrations of Cu in soil solution range from 0.5 to 135 μg L^{-1}, depending on techniques used and on soil types. Gaw (2002) showed that Cu concentrations varied from 7 to 490 mg kg^{-1} in a survey of 43 horticultural soils in the Auckland area, New Zealand. Sites currently being used for orchards had an average concentration of 209 mg kg^{-1} Cu whereas vineyard soils had an average of 105 mg kg^{-1}. Background Cu concentrations in the soils of forest remnants were 10 mg kg^{-1} as reviewed by Cornforth et al. (2003). In the same way, vineyard soils in France, Greece, Portugal, Slovenia and the Czech Republic have been found to contain concentrations in excess of 100 mg kg^{-1}, above which the biological quality of the soils is substantially degraded through a strong effect on microbial action. Excessive concentrations of Cu can hinder topsoil rooting in young vines. Also, there is the risk of Cu accumulating in river water or even river sediments by effect of erosion-induced mass movements in Cu-contaminated soils, resulting in potential environmental problems (Fernández-Calvino et al., 2009a).

2.3.3. Forms and mobility of Cu in soils

Soils usually restrain Cu in many forms depending upon soil pH. In soil solution it may occur as cations: Cu^{2+}, $CuOH^{+}$, $Cu_2(OH)_2^{2+}$ adsorbed on clay minerals, and as anions: $Cu(OH)^{3-}$, $Cu(OH)_4^{2-}$, and $Cu(CO_3)_2^{2-}$ (Kabata-Pendias and Sadurski, 2004). However, Cu hydroxides and carbonates are likely to predominate. Generally, Cu is accumulated in the upper few centimeters of soils; however, due to its tendency to be adsorbed by soil organic matter, carbonates, clay minerals, and oxyhydroxides of Mn and Fe, it may be also accumulated in deeper soil layers. Humic acids are reported to reveal a large binding capacity for this metal (Logan et al., 1997). Cu is a rather immobile element in soils and shows relatively little variation in total contents of soil profiles. The common characteristic of Cu distribution in soil profiles is its accumulation in the top horizons. This phenomenon is an effect of various factors, but above all, Cu concentration in surface soils reflects its bioaccumulation as well as its anthropogenic sources (Kabata-Pendias, 2011). It is reported that, the main variables affecting the Cu mobility in soils include soil organic matter, dissolved organic matter, pH, and Cu soil content. Overall solubility of both cationic and anionic forms of Cu decreases at about pH 7–8 (Ponizovsky et al., 2006). It was estimated also that hydrolysis products of $Cu(CuOH)^{+}$ and $Cu^{2+} + 2(OH)_2$ are the most significant species below pH 7, while above pH 8, anionic hydroxy complexes of Cu become important.

The precipitation of $CuCO_3$ in calcareous soils is a main process affecting the Cu activity in soil solution (Ponizovsky et al., 2007) as reviewed by (Kabata-Pendias, 2011).

Solubility of Cu in soil solution greatly dependents on soil pH and will be most readily available at pH values below 6 (Adriano, 2001). In acidic soils, for example developed on granitic rocks, Cu can migrate throughout soil profiles more easily and thus cause groundwater pollution (Nóvoa-Muñoz et al., 2007). Cu mobility in soils can increase at pH values above ~7.5 due to the solubilization of soil organic matter (SOM) and formation of Cu-SOM complexes (Fernández-Calviño et al., 2009a). In general, Cu in soils is mostly associated with SOM, Fe-, Mn-(hydr)oxides and to a lesser extent with clay minerals through specific and non-specific adsorption (Fernández-Calviño et al., 2009b). However, SOM can influence the mobility of Cu by two different means: while particulate SOM will act as a sorbent for Cu, soluble SOM will actually complex Cu, increasing thus its solubility, especially at alkaline pH (above ~7.5) (Martínez-Villegas and Martínez, 2008). When Cu enters the soil, as a result of the wash-off from plant leaves (Paradelo et al., 2008) and accidental spills of the fungicides, its speciation rapidly changes and Cu is sorbed and co-precipitated in the soil (Komárek et al., 2010). This redistribution of Cu into less available chemical fractions of soils which decreases its mobility and bioavailability is referred to as aging (Sayen et al., 2009) as reviewed by Komárek et al. (2010).

The phytotoxicity of Cu is the highest in acidic soils with a low cation exchange capacity. Cu phytotoxicity to agricultural plants grown on calcareous soils from former vineyards has been observed as well (Michaud et al., 2007). Although Cu concentrations in roots are a good indicator of Cu bioavailability in soils (Chopin et al., 2008), this time consuming approach is not suitable for routine analyses (Komarek et al., 2010). So, it is needed to point out that Cu uptake by roots is species-dependent and influenced by root type and size (i.e., fine vs. coarse roots) (Chopin et al., 2008). Total Cu concentrations alone do not provide adequate information about the bioavailability of the metal in soils as well (Wightwick et al., 2008). On the other hand, water-soluble Cu concentrations alone do not give sufficient information either, because a portion of exchangeable Cu (not extractable by water) in soils can be easily taken up by roots (Komárek et al., 2010).

It is well documented that, Cu reveals a strong affinity for S, hence its principal minerals are chalcopyrite, $CuFeS_2$; bornite, Cu_5FeS_4; chalcocite, Cu_2S; and covellite, CuS. During the weathering of Cu sulfides, Cu is incorporated in oxide and carbonate minerals of which cuprite, Cu_2O; tenarite, CuO; malachite, $Cu_2CO_3(OH)_2$; and azurite, $Cu_2(CO_3)_2(OH)_2$ are the most common. Cu is often associated with sphalerite, ZnS; pyrite, FeS; and galena, PbS. Its ores are commonly found in acid igneous rocks and various sedimentary deposits (Kabata-Pendias, 2011). Cu has a high affinity for peptide and sulphhydryl groups, and thus to cysteine-rich proteins, as well as also for carboxylic and phenolic groups. Therefore, more than 98% of the Cu in plants is present in complexed forms and the concentrations of free Cu^{2+} and Cu^{+} is extremely low in the cytoplasm (Broadley et al., 2012).

Cu is mainly transported into streams and water bodies as runoff either due to natural weathering or to soil disturbances (68 % of the total release of Cu to water). Cu sulfate used in agriculture represented 13 % of the Cu released to water, and urban runoff contributed with 2 % (Perwak et al., 1980). In the absence of specific industrial sources, runoff can be the major factor contributing to Cu pollution in rivers (Nolte, 1988). Input of Cu into aquatic ecosystems mainly through waste discharges into saline waters, industrial discharges into freshwater, and a wood preservative (Nriagu, 1979a) increased sharply during the past century and is two to five times higher than natural loadings (Nriagu, 1979b).

2.3.4. Cu as micronutrient

It is an essential micronutrient, a constituent of the plastocyanin chloroplast protein, and a component of the electron transport system linking photosystems I and II in the photosynthetic process. Cu participates in protein and carbohydrate metabolism and N_2 fixation. It is a component of some enzymes that reduce atoms of molecular oxygen such cytochrome oxidase, ascorbic acid oxidase, and polyphenol oxidase (Broadley et al., 2012; Jones, 2005). Cu is a transition element with roles in photosynthesis, respiration, C and N metabolism, and protection against oxidative stress. It forms highly stable complexes and participates in electron transfer reactions like Fe. Divalent Cu is reduced readily to monovalent Cu which is unstable (Broadley et al., 2012). It is an essential micronutrient, a constituent of the plastocyanin chloroplast protein, and a component of the electron transport system linking photosystems I and II in the photosynthetic process. Cu participates in protein and carbohydrate metabolism and N_2 fixation. It is a component of enzymes that reduce atoms of molecular oxygen (cytochrome oxidase, ascorbic acid oxidase, and polyphenol oxidase) and is involved in the desaturation and hydroxylation of fatty acids (Jones, 2003). That means most of the functions of Cu as a plant nutrient are based on enzymatically bound Cu which catalyses redox reactions. In redox reactions of the terminal oxidases, Cu enzymes react directly with molecular oxygen. Terminal oxidation in living cells is therefore catalysed by Cu and not by Fe (Broadley et al., 2012). Due to versatile properties, Cu has a wide range of applications. It is used for the production of various conductor materials such as fertilizers and pesticides. Due to its bacteriostatic properties, it is added to animal fodder (Kabata-Pendias, 2011). The use of Cu-based fungicides in vineyard soils is widely documented worldwide.

Cu is an essential micronutrient and at the same time it is an extremely toxic element in high levels as a consequence of its redox properties (Yruela, 2005), adequate Cu concentrations are needed for better plant growth and development. Cu deficiency symptoms appear if the Cu level in growth medium in below range of 10^{-14} – 10^{-16} M, where Cu content in plant tissues is 10 $\mu g\ g^{-1}$ (Baker and Senef, 1995). Cu concentration in soil solution ranges between 10^{-6} – 10^{-9} M (Kabata-Pendias, 2011), thus plants have to maintain a suitable concentration of the metal in case of low or high levels of the metal in soil solution. Cu alters photosynthetic electron transport both at too high or too low levels, and consequently plants must carefully control the uptake of Cu from the soil, the transport, the distributed and the compartmentalization within their various tissues. Accordingly, plants have homeostatic mechanisms to maintain the appropriate Cu concentration (Yruela, 2005). Elevated Cu levels in plants can cause increased antioxidant responses, especially in leaves and roots being both Cu-dependent, as a consequence for increasing oxidative stress in plant due to increased production of highly toxic oxygen free radicals (Halliwell and Gutteridge, 1984). Macnair et al. (2000) defined the tolerance of plants to potential toxic metals as ability of plant to survive in soils that are toxic to other plants.

There are different possible mechanisms that plants can have at the cellular level in order to tolerate high metal concentrations in growth medium. These mechanisms are represented primarily by avoidance of uptake and accumulation of toxic metals within sensitive parts of the plant. The potential cellular mechanisms involved in tolerance include those involving I) reduction of metal uptake; II) stimulation of the efflux pumping of the metal at the plasma membrane; III) chelating of metals by phytochelatins; IV) compartmentation of metals in the vacuole (Hall, 2002). Moreover, adding excess iron in growth medium might mitigate toxicity symptoms of Cu as a result of the competition between Cu and Fe at the root level (Bernal et al., 2004).

2.3.5. Cu in plants

Cu is essential for the normal growth and metabolism of all living organisms (Schroeder et al., 1966). It functions as a catalyst in photosynthesis and respiration. It is a constituent of several vital enzymes involved in building and converting amino acids to proteins. Cu is important in carbohydrate and nitrate metabolism. It is essential to the formation of lignin in plant cell walls which contributes to the structural strength of the cells and the plant. It also affects water permeability, reproduction, and disease resistance (Kabata-Pendias, 2011). In soil, Cu is the most immobile micronutrient. Therefore, Cu uptake is strongly correlated with root systems, where vigorous root systems take up more Cu. Initially, Cu is equally distributed in the plant but is relatively immobile. Moreover, for better plant growth, adequate Cu supply is needed. Only trace amounts of Cu are required for plant nutrition; however, at high concentrations, Cu can be potentially toxic. Critical deficiency levels of Cu are in the range of 1–5 mg kg^{-1} plant dry mass, and the threshold for toxicity is above 20–30 mg kg^{-1} dry mass (Marschner, 1995). The amount of Cu contained in healthy plants varies considerably within this range and depends both on the species and the Cu-feeding status. Typically, symptoms of deficiency start when Cu decreases below 5 μg g^{-1} DW in vegetative tissues, while toxicity levels are observed above 20-30 μg g^{-1} DW or higher in the same tissue (Marschner, 2002). However, it may be generally stated that total Cu contents below 10 mg kg^{-1} in different soils may indicate deficiency as reviewed by Kabata-Pendias (2011). Otherwise, poor growth, delayed flowering, and plant sterility are the foremost symptoms of insufficient Cu supply. Moreover, Cu deficiency in plant growth may appear as wilting with leaf tips turning a bluish-green color. In cereals plants, the tips may become brown and appear to mimic frost damage. Additionally, young tissues show chlorosis, distortion, and necrosis. The death of the growing points often leads to excessive tillering in cereal crops and excessive branching in dicots (Yruela, 2005).

Although soil contains low Cu concentrations, especially soluble forms for plants, Cu toxicity can occur as a consequence for increased use of fungicides and agrochemicals in agricultural production (Elhawat et al., 2013). Under toxic Cu levels, plants appear stunted and are usually bluish in color, and eventually turn yellow or brown. Also, seed germination, plant vigor and iron intake are reduced. Furthermore, Cu-induced chlorosis and root malformation are the most common characteristic symptoms of this toxicity (Maksymiec and Krupa, 2007).

Toxic levels of Cu occur naturally in some soils whereas others may contain high levels of Cu as a result of the anthropogenic activities such as mining, smelting, manufacturing, agriculture, and waste disposal techniques which release trace elements into the environment. Cu fungicide inputs have been reported as the main cause of high concentrations of Cu in soils. Applied Cu from different agrochemical sources such Cu-fungicides to agro-environment may be adsorbed and are transported to the groundwater and pollute it besides polluting the soils (Elhawat et al., 2013).

2.3.6. Forms and accumulation of Cu in plants

It is well know that the difference between optimum and toxic concentrations of Cu in many economically important crops is very small. Normal and phytotoxic concentrations of Cu in plant leaves are 5-20 and 20-300 mg kg^{-1}, respectively although there are some species variations (Alloway, 1995). Despite this, examples of Cu toxicity in plants are rare (Cornforth et al., 2003). The proportion of total soil Cu which is available to plants depends on soil composition and pH. Cu is held strongly in soils containing relatively large concentrations of organic material, clay, or oxides of Fe, Al, and Mn. These forms are largely unavailable to plants in the short term but may become slowly available if the more plant-available fractions are depleted (Cornforth et al., 2003). Availability is also greater in acid conditions although the link between pH and Cu availability is less marked than for other heavy metals. Liming an acid soil increases the amount of Cu adsorbed but does not necessarily decrease the amount of plant available Cu proportionally. This is because lime may increase the amount of Cu released by mineralisation of organic Cu (James and Barrow, 1981) as reviewed by Cornforth et al. (2003).

It is well documented that the bioavailability of soluble forms of Cu depends most probably on both the molecular weight of Cu complexes and on the amounts present. Compounds of a low molecular weight liberated during decay of plant and animal residues as well as those applied with sewage sludge may greatly increase the availability of Cu to plants (Kabata-Pendias, 2011). It should be also emphasized, however, that concentrations of Cu in soil solutions are principally controlled by both the reactions of Cu with active groups at the surface of the solid phase and by reactions of Cu with specific substances. The behavior, phytobioavailability and toxicity of Cu are influenced by its species, and are not a function of its total concentration (Allen, 1993). Several soil variables control the Cu solubility and thus bioavailability; these include: pH, oxidation and reduction potential, soil organic matter, soil texture, mineral composition, temperature, and water regime. The mobility of Cu is especially reduced at the presence of large mineral colloids with Fe–Al-oxyhydroxide coatings, by oxyhydroxide particles of Al, Mn, Fe, and by organic matter (Kabata-Pendias and Sadurski, 2004).

The affinity of Cu to separate soil fractions decreases in the following order: Mn-(hydr) oxides > SOM > Fe-(hydr)oxides > clay minerals (Bradl, 2004). Dissolved organic matter has a great affinity to fix Cu and thus to inhibit its sorption in soils. These phenomena are attributed to the formation of soluble Cu-organic complexes (Zhou and Wong, 2003). In mineral soils, natural attenuation of Cu occurs as an effect of Cu substituting for Ca in calcites present in calcareous soils and as precipitation of $Cu(OH)_2$ and/or $Cu_2(OH)_2CO_3$ in other soils (Ma et al., 2006). It was observed that the long-term application of Cu fertilizers resulted in a great Cu accumulation in surface soil (0–15 cm) due to its low mobility, since a great proportion of added Cu was bound to mineral soil fractions (Wei et al., 2007). The more readily plant available forms include Cu ions in solution, soluble organic-Cu complexes, and Cu on the soil's exchange complex (Alloway, 1995). However, Bolan et al., (2003) distinguish between Cu adsorbed on soluble organic matter and free Cu^{2+} ions in the soil solution: the former may increase the mobility of Cu in the soil while the latter represents the plant available fraction (Cornforth et al., 2003).

It is reported that soil carbonates proved to be another important factor controlling Cu mobility (and thus bioavailability) in soils. It is also found that, the activity of Cu in calcareous soils is to a great extent controlled by the surface precipitation of $CuCO_3$ (Ponizovsky et al., 2007). This is especially important in alkaline soils containing high concentrations of carbonates, which is the case for many vineyards. The retention of Cu in calcareous soils through co-precipitation with carbonates is associated with the release of Ca^{2+}, Mg^{2+}, Na^+ and H^+ into the soil solution at equimolar ratios (Ponizovsky et al., 2007). The precipitation of newly formed Cu phases in the soil presents thus an important retention mechanism of Cu retention in soils. The solubility of Cu minerals in soils decreases in the following order: $CuCO_3$ > $Cu_3(OH)_2(CO_3)_2$ (azurite) > $Cu(OH)_2$ > $Cu_2(OH)_2CO_3$ (malachite) > CuO (tenorite) > $CuFe_2O_4$ (cupric ferrite). Copper sulfates, such as $CuSO_4$ (chalcocyanite) and $CuSO_4 \cdot 5H_2O$ (chalcanthite), are highly soluble and require very high Cu concentrations to form in soils (Lindsay, 1979) as reviwed by Komárek et al. (2010).

In excessive quantities Cu becomes toxic as it interferes with photosynthetic and respiratory processes, protein synthesis and development of plant organelles (Upadhyay and Panda, 2009). Specifically excess Cu can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability, leading to ion leakage (Bouazizi et al., 2010). Reports are also available on induced deficiency of various minerals content under Cu toxicity (Lequeux et al., 2010). Apart from this, the information on plant metabolism is sporadic. Hence, efforts have been made to establish the toxic level of Cu on mung bean plants. It is found that, low Cu concentration (50 mg kg^{-1}) had stimulatory effect on growth, dry matter yield and mineral nutrient content of mung bean. Whereas, Cu application beyond these levels (100-250 mg kg^{-1}) adversely affected the growth, dry matter yield and nutrient content (Manivasagaperumal et al., 2011).

Cu can be present in several insoluble forms; (1) adsorbed on surface of metal oxides, clay minerals, humic substances and organo-mineral complexes, (2) in structure of secondary minerals or in amorphous iron and manganese oxides; (3) or associated with antigenic sulfides. Gunkel et al. (2003) found that applied Cu was mostly concentrated in the MnO_x, FeO_x and organic soil fractions. Although the total Cu levels were quite low on Cu-enriched plots, ranging from 68–135 mg kg^{-1}, anthropogenic Cu in soil influenced the maize crop yield. The recommended method for determine the real bioavailability nowadays is the sequential extraction method and the plant bioassay.

Most of the cases of hyperaccumulation of Cu and Co have been reported from the metalliferous soils of the Democratic Republic of Congo (formerly Zaire), where the two metals occur together at elevated levels in the soils, but in widely varying proportions (Reeves, 2006). A few other records of plants with > 1000 mg kg^{-1} Cu from Cu-mineralized areas (Dykeman and De Sousa, 1966) should be reinvestigated in detail. Normal concentrations of Co and Cu in plants are in the ranges 0.03-2 and 5-25 mg kg^{-1}, respectively. The tupelo or black gum of the southeastern United States (*Nyssa sylvatica*) is remarkable in being able to accumulate as much as 845 mg kg^{-1} Cu from normal soils (Brooks, 1977). Plant Cu concentrations are controlled within a remarkably narrow range, and Cu concentrations above 100 mg kg^{-1} are rarely found in carefully washed plant leaves, even in the presence of high soil Cu. The Cu hyperaccumulators have been found in more than a dozen plant families (Reeves and Baker, 2000). It should also be noted that most of the Cu accumulators are not restricted to metalliferous soils. Therefore, great care needs to be taken in selecting seed of any of these species for studies connected with metal-accumulation experiments or with studies of their potential for phytoremediation (Reeves, 2006).

2.3.7. Impacts of Cu-agrochemicals on Cu levels in soils

Contamination of agricultural lands with Cu has been accelerated due to its wide and repeated use in agriculture as fertilizers or fungicides to protect vines, citrus trees, and other crops against fungus diseases (He et al., 2005). Due to a long history of citrus or vineyard production, Cu has accumulated in the soils. A large proportion of soils under citrus production contains total Cu content above 85 mg kg^{-1}, the critical level for ecosystem health (Schuler and Hoang, 2008). Soil contamination with Cu causes soil degradation, Cu phytotoxicity, and increased transport of Cu to surface and ground waters (He et al., 2010).

As mentioned before due to its versatile properties, Cu has a wide range of applications. Cu is widely used in agriculture as fertilizers, pesticides etc. Also, due to its bacteriostatic properties, it is also used as a feed additive in livestock and poultry nutrition (Kabata-Pendias, 2011). Soil contamination by Cu compounds has been the subject of detailed studies for several decades and a large database has been already collected and presented in a number of monographs and papers. Several significant sources such as fertilizers, sewage sludge, manures, agrochemicals, industrial by-product wastes and the quality of irrigation waters have contributed to increased Cu levels to agricultural soils (Kabata-Pendias and Mukherjee, 2007).

It is well documented that the most important sources of Cu contamination include old mining area, deactivated Cu ore plant, nonferric metal mining, metal-processing industry, urban gardens, orchards, and parks, sludged, irrigated, or fertilized farmland, application of fungicides, vineyard soils, and military shooting range (Kabata-Pendias, 2011). Dry and wet deposition around mining and smelting sites, wastewater irrigation, compost application, including municipal waste, sewage sludge or their combination, and spraying of heavy metal-containing pesticides or herbicides have been reported to contribute to the input of anthropogenic Cu and other heavy metals into agricultural soils (Yu et al., 2004).

It is well known that Cu-based fungicides (such as the Bordeaux mixture, $CuSO_4$ + $Ca(OH)_2$) have been intensively used in Europe since the end of the 19th century to control vine (*Vitis vinifera* L.) fungal diseases, such as downy mildew caused by *Plasmopara viticola*. Besides vineyards, Cu-based fungicides have also been extensively used such as in hop fields (Komárek et al., 2009), coffee (Loland and Singh, 2004), apple (Li et al., 2005), avocado orchards (Van Zwieten et al., 2004) and during the cultivation of several vegetables such as tomatoes and potatoes (Adriano, 2001) as reviewed by Komárek et al. (2010). However, intensive and long term use of these fungicides in agricultural production has increased soil Cu concentrations and this is likely most pronounced in vineyards (Komárek et al., 2010). Currently, EC regulation 473/2002 (EC, 2002) restricts the annual dose of applied Cu to 6 kg Cu ha^{-1}, which corresponds to an annual accumulation of about 5 mg Cu kg^{-1} soil in the top 10 cm assuming no losses (Ruyters et al., 2013). Such a sustained application for more than 150 years would have increased soil Cu concentrations to 750 mg Cu kg^{-1} in unplowed vineyard soils, whereas plowing the first 30 cm after removing old vines (every 30–50 years) would yield a topsoil Cu concentrations of about 250 mg Cu kg^{-1}. Measured Cu concentrations in vineyard soils range from 77 up to 3200 mg Cu kg^{-1} (Komárek et al., 2010) and are above legislative limits affecting the sustainability and potentially the productivity of these agroecosystems (Komárek et al., 2010) as reviewed by Ruyters et al. (2013).

2.3.8. Effects of Cu on soil organisms

It is found there were a number of basic criteria that a microbiological property might be expected to fulfill as an indicator in monitoring soil pollution by metals or other pollutants (Brookes, 1995). Soil microbial biomass is considered to be a transformation agent of soil organic materials and a labile pool for plant nutrients. Hence, the change of the soil microbial biomass could lead to a change in the rate of nutrient cycling and the size of the nutrient pool. It is suggested that, soil C mineralization and linked parameters such as CO_2–C production per unit biomass C and unit time (biomass specific respiration rate) might be useful as indicators for the change of soil function (Brookes, 1995). Soil enzyme activities are also useful for detecting changes in soil quality, as they underpin nutrient cycling, and also function as signals of altered microbial community structure caused by environmental impact (Wang et al., 2009).

Because of the human and environmental health hazards that may arise from this pollution, setting soil-Cu limits and adequate management practices are necessary to reach sustainability in this type of crop. High Cu contents can harm soil microbial communities, which are the main agents responsible for long-term sustainability of soil ecosystems (Nannipieri et al., 2003). Some studies have evaluated the effects of Cu on enzymatic activities (Fernández-Calviño et al., 2010a), microbial community structure (Fernández-Calviño et al., 2010b), and bacterial community tolerance (Fernández-Calviño et al., 2011a) in vineyard soils. However, the establishment of limits for Cu accumulation using microbial indicators is difficult because of the confounding effects of soil factors such as texture, pH, and organic matter content (Fernández-Calviño et al., 2011b) as reviewed by (Soler-Rovira et al., 2013).

It is reported that toxic effects of Cu on the microbial communities in vineyard soils have been observed. Enzyme activities in soil were affected at and above total concentrations of 150–200 mg Cu kg^{-1} soil (Fernandez-Calvino et al., 2010a) and nitrification was impaired in soils contaminated up to 380 mg Cu kg^{-1} (Baroux, 1972). Evidence for increased Cu tolerance of the microbial community in response to the Cu contamination has also been reported in such soils (Diaz-Ravina et al., 2007) as reviewed by Ruyters et al. (2013). On the other hand, there is evidence that earthworms (Martin, 1986) and soil microorganisms (Aoyama and Nagumo, 1997) are less active in

Cu-rich soils than in those with concentrations of Cu in the normal range (2-30 mg kg^{-1}) (Cornforth et al., 2003). Earthworm populations have been proposed as indicators of the degree of Cu contamination in orchard and vineyard soils (Paoletti et al., 1998). This approach assumes that earthworms and higher plants are similar in their sensitivity to Cu or that decreased earthworm activity will damage soil structure to the extent that plant growth suffers (Cornforth et al., 2003).

2.3.9. Environmental risks of elevated Cu concentrations

The intensive and long-term use of Cu salts promoted, in viticulture over the years, Cu accumulation in soils. Cu may be toxic for aquatic and soil organisms, bacteria, fungi and plants it also has a negative effect on human health (Turnlund et al., 2004). In soil, Cu is restricted mainly in the top layer because of its ability to tightly bind with carbonates, clay minerals, hydrous oxides of Al, Fe and Mn and organic matter (Mengel et al., 2001). Despite its environmental and agricultural importance, the concentration, distribution and fractionation of anthropogenic, and naturally occurring, Cu in soils is poorly known. Although the total Cu content in soils is a useful indicator of soil deficiency and/or contamination, it does not provide enough information about its environmental impact (Pietrzaka and McPhail, 2004). Sorption on SOM by means of complexation especially with humic and fulvic acids presents possibly the most important retention mechanism for Cu in soils (Strawn and Baker, 2009). Its association with SOM through inner-sphere complexation (e.g., bidentate inner-sphere coordination with carboxyl or amine ligands (Strawn and Baker, 2008)) results in its lower toxicity compared to free Cu^{2+} (Karlsson et al., 2006). Additionally, Cu-rich SOM is less vulnerable to biodegradation (Parat et al., 2002). In most cases, the sorption of Cu in soils follows well either the Langmuir or the Freundlich isotherm as reviewed by Komárek et al. (2010).

Contents of Cu are closely associated with soil texture and usually are the lowest in light sandy soils and the highest in loamy soils. It was found that, Cu toxicity seems to be related to soil texture and a toxicity threshold was established only in light textured soils, for nonbearing potted grapevine and pear plants at a concentration of DTPA-extractable Cu > 141 and 350 mg kg^{-1}, respectively, while in clay-loam soils, both the fruit species showed the possibility to tolerate levels of DTPA-extractable Cu as high as 1000 mg kg^{-1} with no symptoms on shoot growth (Toselli et al., 2009). Cu availability to biota (as a nutrient or toxin) and its mobility are the most important factors to be considered when assessing its effect on the soil environment. Since Cu bioavailability is influenced not only by soil physical and chemical properties, but also by environmental factors such as climate, biological population, and type and source of contaminants, correlation between total and bioavailable Cu cannot be predicted accurately (Pietrzaka and McPhail, 2004).

Cu must be absorbed in small amounts on a daily basis to maintain good health. A daily dietary intake of 1–2 mg is required. However, high levels of Cu can be harmful to health. Inhaling high levels can cause irritation to the nasal passages, mouth, eyes and throat, and ingesting high Cu concentrations can lead to nausea, vomiting and diarrhoea. Exposure to very high levels can damage the liver and kidneys and may lead to death. Cu is classified as a hazardous substance. An excess of Cu may result in Wilson's disease, mostly ending in death (Sharma et al., 2009).

Fungicides have received little attention compared with other types of pesticides, such as insecticides and herbicides, despite the likely environmental risks (Wightwick et al., 2010). Ecological risks of chemicals detected in the environment are often derived by comparisons with environmental quality values and reported ecotoxicological effects values for key sentinel aquatic species (Wightwick et al., 2012). The paucity of ecotoxicological data for fungicides is surprising given their frequency of use and the fact that most do not have specific modes of action and thus is likely to be toxic to a wide range of organisms, not just fungi (Maltby et al., 2009). There is a widespread presence of residues of many different organic fungicide compounds in the surface waters of a horticultural-production catchment. Whilst it appears that the fungicides detected are likely to pose a low ecological risk to fish, aquatic invertebrates, and algae, it is important to note that at present there are few ecotoxicological data available describing the effects of fungicides on aquatic fungi and microorganisms despite the fact that these organisms are likely sensitive to fungicides and play a key role in aquatic ecosystems (e.g., decomposition and nutrient cycling) (Milenkovski et al., 2010) as reviewed by Wightwick et al. (2012).

3. MATERIALS AND METHODS

3.1. Source of plant material

Four ecotypes of giant reed were tested in the present work. All ecotypes had almost similar morphological features.

- Blossom (BL) ecotype was brought from South Carolina, USA.
- 20SZ and STM ecotypes were brought from Újszentmargita village, Hungary (Pro-Team Co. Ltd.).
- ESP ecotype was brought from Spain (Biotek Co Ltd, K-12)

All plant materials used for the current study were somatic embryo-derived plantlets of giant reed (*Arundo donax* L.) according to patent application of the University of South Carolina (Márton and Czakó, 2002). Embryogenic callus culture of the BL ecotype was originally obtained from the University of South Carolina, while other ecotypes were obtained from MOP Biotech Co. Ltd. (Nyíregyháza, Hungary). All ecotypes were propagated in the Ottó Orsós Laboratory, Department of Plant Biotechnology, University of Debrecen, Debrecen, Hungary.

3.2. *In vitro* experiments

3.2.1. Experiment 1: growing giant reed on liquid medium

An *in vitro* experiment was carried out in order to investigate the efficiency of giant reed ecotypes (BL and 20SZ) grown on MS medium (Murashige and Skoog, 1962) to remove Cu from artificially Cu-contaminated water bodies and monitoring impacts of Cu on growth dynamics of giant reed ecotypes. Two identical size aseptic seedlings (5 cm height) were grown on MS medium (Murashige and Skoog, 1962) supplemented with increasing concentrations of Cu under aseptic conditions as described by Márton and Czakó (2004; 2007).

3.2.1.1. Experiment layout and growth conditions

The randomized complete block design (RCBD) was used for experiment layout with five replications, each having two plants per tube. The treatments included control (no Cu) and six doses of copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) i.e., 0, 1, 2, 3, 5, 10, and 26.8 mg L^{-1} Cu. Two giant reed plantlets of BL and 20SZ ecotypes almost equal morphological characteristics were directly transplanted to autoclaved glass tubes (2.5 × 25 cm) containing MS medium as described by Márton and Czakó (2004; 2007) for 6 weeks under gnotobiotic conditions. The cultivated tubes were set under white fluorescent lamps (41 µmol m^2 s^{-1} photon flux density), and 16/8 h light/dark cycle and temperatures of 30 ºC during the day and 27 ºC during the night. The average relative humidity was recorded to be 75%.

3.2.2. Experiment 2: growing giant reed on solidified medium

The main research objectives of this *in vitro* experiment were to assess the possible utilization of Cu-contaminated soils for biomass production, monitor the growth performance of giant reed ecotypes (BL and 20SZ) under increasing Cu concentrations growing on solid medium as simulated model for agricultural soil, and determine the bioaccumulation and transportation of Cu by giant reed plants.

3.2.2.1. Experiment design

A randomized complete block design (RCBD) was used for experimental layout with three replications, each having five plants per autoclaved 1-L glass jar containing 150 ml of MS medium (Murashige and Skoog, 1962). 20 gram agar per liter was added with aim to solidify the medium as described by Márton and Czakó (2004; 2007). The treatments included control (no Cu) and six doses of Cu using copper (II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) i.e., 0, 1, 2, 3, 5, 10 and 26.8 mg Cu L^{-1}. Five giant reed seedlings of both BL and 20SZ ecotypes of uniform morphological characteristics were transplanted into solidified nutrient solution for six weeks under aseptic conditions. The cultivated jars were set under 16/8 hrs light/dark cycle and temperatures of 30 ºC during the day and 27ºC during the night. The average relative humidity was recorded as 75%.

3.2.3. Phytoextraction indices

The ability of giant reed ecotypes to transport and accumulate Cu in different plant parts (roots, culm, and leaf blade) was assessed using the bioconcentration factor (BCF) (Chamberlain, 1983; Harrison et al., 1989), the transportation factor (TF) (Barman et al., 2000), , the bioaccumulation percent (BAP), and removal rate (RR) as follows:

BCF = [Cu]plant//[Cu]growth medium

TF = [Cu]shoot/[Cu]root

BAP = [Cu in plant part (root, culm or leaf blade)] / [Cu in total plant] × 100

RR = [Cu in growth medium before − Cu in growth medium after] / [Cu in growth medium before]

3.2.4. Copper analysis

Before metal analysis, plants were washed carefully with deionized water to remove any adhering Cu to plant parts. After air drying, plants were divided into roots, culms, and leaf blades. All plant parts were wet digested in 5 mL concentrated HNO_3 and 1.5 ml H_2O_2 then diluted to 25 ml using ultrapure water (Page, 1982). Cu concentration was measured using atomic absorption spectrophotometer (AAS, PERKIN ELMER 3300, USA) with a detection limit of 100 ppb.

3.2.5. Growth parameters and yield data

Different vegetative parameters of both ecotypes BL and 20SZ of giant reed studied under Cu stress including average shoot and root length, wet and dry weight of various plant parts including root, culm, and leaf blade, number of new tillers per plant, and toxicity symptoms after the Cu treatments were measured at the end of experiment. The values are average of ten plants per treatment.

3.3. *Ex vitro* experiment

A greenhouse experiment was carried out, between July and October 2013, using 1-kg plastic pots in order to assess growth effeciency of three ecotypes of biomass candidate giant reed (BL, STM, and ESP) growing on elevated Cu concentrations. In addition, biomass production of giant reed under high Cu levels was evaluated with aim to determine the possible directing of Cu-contaminated soils towards renewable energy production during the phytoremediation process as additional benefit. A randomized complete block design (RCBD) was used for experimental design with five replications. Two identical (5 cm height) and aseptic seedlings of giant reed ecotypes were transplanted in 1-kg plastic pot.

3.3.1. Experiment installation and treatments

Sand (quartz, SiO_2) was used as growth medium (soil-like growth medium) for this research. Sand was bought from Hungarian market (producer Aquabau Magyarország Kft., seller Buamax Company, Debrecen, Hungary). Sand was washed with diluted HCl and then rinsed many times in distilled water. No nutriets were detected in HCl-washed sample of sand. Each plastic pot was filled up with almost 1 kg of air-dry sand. Copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) was used to prepare the treatments of Cu. Four different levels of Cu were applied, i.e., 100, 200, 300, and 400 mg Cu kg^{-1} against control (no Cu). All pots were received Cu treatments and then irrigated by distilled water to reach its saturation percent and left to the next day to attend equilibrium. In the morning, two plantlets of studied giant reed ecotypes were treansplanted per each pot and then all experimental pots were kept under foliar tent in order to acclimatize the seedlings with greenhouse conditions. During the whole experimental period all pots were kept at its water holding capacity (WHC) (almost 75% of its saturation percent) using distilled water in order to prevent the leakage of Cu from the pot. The following nutrients were added to experimental pots; $(NH_4)_2SO_4$ 10 mg kg^{-1}; $Na_2HPO_4{\cdot}12H_2O$ 10 mg kg^{-1}; K_2HPO_4 10 mg kg^{-1}; $CaCl{\cdot}2H_2O$ 2.6 mg kg^{-1}; $MgCl_2{\cdot}6H_2O$ 2 mg kg^{-1}; $FeCl_3{\cdot}6H_2O$ 0.5 mg kg^{-1}; $MnSO_4{\cdot}H_2O$ 0.1 mg kg^{-1}. The experiment started in July 2013 and was harvested by beginning of November. Number of new buds, length of plant, and number of leaves were recorded twice, after 6 and 10 weeks from plantation, during the experiment period.

3.3.2. Vegetative parameters

Vegetative parameters of BL, STM and ESP ecotypes of giant reed studied under Cu stress including average shoot and root length, wet and dry weight of various plant parts including root, culm, and leaf blade, number of new tillers per plant, and toxicity symptoms after the Cu treatments were measured at the end of experiment. During experiment after 6 and 10 weeks from starting the experiment, number of new tillers, length of shoot part and number of leaves were recorded. At the end of experiment number of new tillers, internodes and leaves was counted. Length of internode, shoot, and root also measured. Volume of root and shoot systems was volumetrically measured using cylinder filled with distilled water to certain volume then shoot and root system separately immersed into the cylinder and the increase in water volume represented volume of shoot or root system.

3.3.3. Copper analysis in plant

Before Cu analysis, plants were washed carefully with deionized water to remove any adhering Cu to plant parts. After air drying, plants were divided into roots, culms, and leaf blades. All plant parts were wet digested in 5 mL concentrated HNO_3 and 1.5 ml H_2O_2 then diluted to 25 ml using ultrapure water (Page, 1982). Cu concentration was measured using Atomic Absorption Spectrophotometer (AAS, PERKIN ELMER 3300, USA) with a detection limit of 100 ppb.

3.3.4. Copper measurement in soil-like growth medium

Total content of Cu in soil samples were determined by digesting 0.5 g sample using a mixture of 1 ml 60 % perchloric acid, 5 ml concentrated nitric acid and 0.5 ml concentrated sulfuric acid according to Stewart (1989). Available Cu was determined using 0.01 M $CaCl_2$. Five grams of soil-like growth medium were mixed with 50 ml of $CaCl_2$ 0.01 M and shaken horizontally for 72 h (Van Ranst et al., 1999). Cu concentrations were measured by atomic absorption spectrophotometer (AAS, PERKIN ELMER 3300, USA) with a detection limit of 100 ppb.

3.3.5. Determination of total soluble protein content and MDA content

Total soluble protein content in root, culm and leaf blade of BL, STM and ESP plants was measured by the method of Bradford (1976). The lipid peroxidation (LPO) was determined from root, culm, and leaf blade by the method of Zhang and Huang (2013) by measuring the amount of malondialdehyde (MDA). The leaf tissues (~ 100 mg) were homogenized in 1 ml 0.1% (w/v) trichloroacetic acid (TCA) solution using cold mortar and pestle. The homogenates were centrifuged at 10.000 x g for 10 min. Then 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA solution was added into 1 ml of supernatant and incubated at 96 °C for 30 min. The tubes were cooled by transferring into an ice bath. The absorbance of the supernatant was recorded at 532 nm. Standard curve was generated from MDA standard. The concentration of MDA of giant reed ecotypes samples was calculated from absorbance knowing calibration curve.

3.3.6. Photosynthetic pigment determination

The photosynthetic pigment content of giant reed leaves were measured by spectrophotometric method based on Porra et al. (1989). For the sample preparation leaf disc was cut from giant reed and the chlorophyll content was extracted by N'N dimethyl-formamide (HPLC grade, Scharlab S. L., Spain) overnight. The absorbance was measured by spectrophotometer (Amersham Biosciences Ultrospec 2100 Pro UV/Visible, Biochrom Ltd. Cambridge CB4 0FJ, England) on 663 and 645 nm wavelength and from these data the chlorophylls *a* and *b*, and carotenoids were calculated.

3.3.7. Measurement of fast chlorophyll *a* fluorescence transient

The fast chl *a* fluorescence transient measurement were carried out with Handy PEA fluorometer (Hansatech Instruments Ltd, Norfolk, UK). Dark adapted (30 min) intact tobacco leaves were illuminated with continuous light (650 nm peak wavelength, 300 µmol photons m^2 s^{-1} maximum light intensity). Light is provided by an array of three red LEDs focused on a circle of 5 mm diameter of the sample surface. Values for maximum fluorescence (Fm) and ground fluorescence (F0) from the fluorescence induction curve (OJIP) were used for calculation of the Fv/Fm ratio.

3.3.8. CO_2 assimilation by infrared gas analyzer (LICOR)

The net photosynthesis rate was measured by the LICOR LI-6400 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA) at room temperature. It has two infrared gas analyzers to measure CO_2 and H_2O mole fraction in air. Light was controlled and stable in the sample chamber. The photosynthetic active radiation (PAR) was 865 µmol photon m^{-2} s^{-1}, with 90 % red (630 nm) and 10 % blue (470 nm) light. We measured six times per leaf, in two repetitions.

3.4. Data analysis

Data analysis was performed using Microsoft Excel 2003 (mean values and standard deviation) from three individual experiments, and the statistical analysis was conducted using the SPSS 13.0 software package (SPSS Inc., Chicago, IL). The data obtained were subjected to two-way analysis of variance (ANOVA) for assessing the significance of quantitative changes in the variables as a result of Cu treatments and their respective interaction with plant types. When a significant difference was observed between treatments, multiple comparisons were made by the Duncan's test. Significant differences were accepted at the level $p < 0.05$.

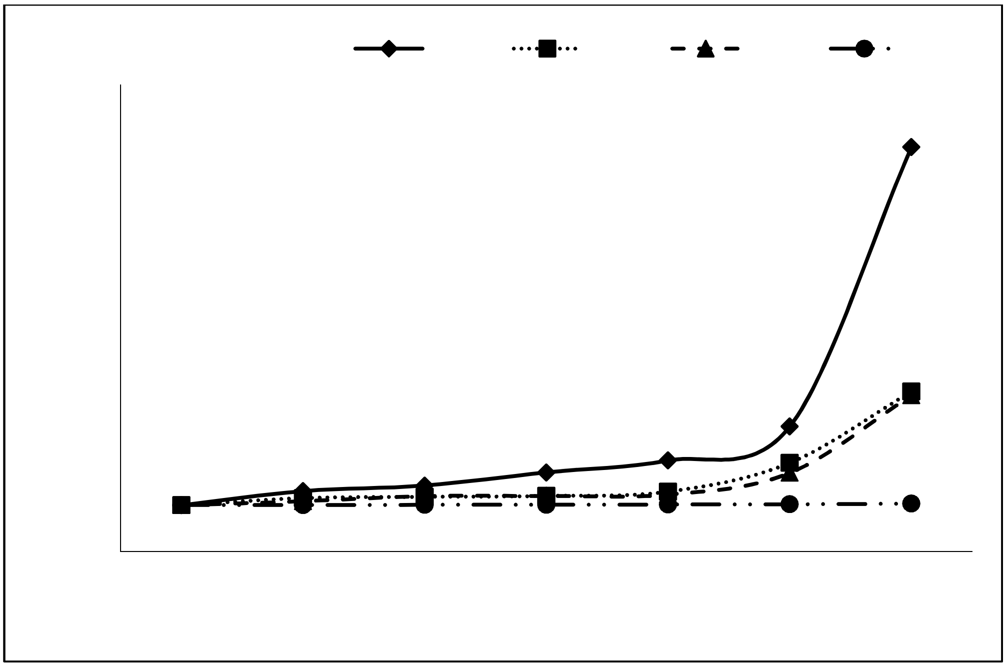
4. RESULTS AND DISCUSSION

4.1. *In vitro* experiments

4.1.1. Experiment 1: growing giant reed ecotypes on liquid medium

4.1.1.1. Cu transport to giant reed plants

In general, removal of metals in contaminated water bodies may occur through a number of processes, including sedimentation/coagulation, filtration, plant uptake/removal efficiency, adsorption, formation of solid compounds, cation exchange, and microbial-mediated reaction, especially oxidation (Pei-ying et al., 2010). The results of Cu concentrations determined in the different parts of the plant and in the growth medium are presented in Fig. 1. After growing both BL and 20SZ ecotypes of giant reed (*Arundo donax* L.) in different Cu treatments for 6 weeks, along with increase Cu concentration in culture medium, Cu content (mg kg^{-1}) in various plant parts linearly increased. At lowest Cu concentration (1 mg L^{-1}), Cu content in root, culm, and leaves was 2.99, 1.50, and 0.84 for BL ecotype and 0.41, 0.16, and 0.19 for 20SZ ecotype. At 26.8 mg L^{-1} treatment, Cu content in root, culm, and leaves of BL plants was 76.57, 24.28, and 23.44 µg g^{-1} DM, while 55.91, 69.49, and 10.18 µg g^{-1} DM were measured in root, culm, and leaves of 20SZ plants, respectively. Moreover, for Cu accumulation in different plant parts, both ecotypes BL and 20SZ showed the same tendency where the order of Cu accumulation was: nutrient solution < leaf blade < culm < roots. Very small fluctuation was found for 20SZ ecotype where, at the highest Cu treatment (26.8 mg L^{-1}), significantly, the culm was able to accumulate Cu higher than root 69.49 and 55.91 mg kg^{-1}, respectively. Cu left over in growth medium was 0.02, 0.07, 0.08, 0.14, 0.18, and 0.33 mg L^{-1} for BL ecotype and 0.00, 0.00, 0.00, 0.00, 0.30, and 0.55 mg L^{-1} for 20SZ ecotype for Cu treatments of 1, 2, 3, 5, 10 and 26.8 mg L^{-1}, respectively. The mass balance remained maintained for both ecotypes, which was in agreement with Wu Qi et al. (2012).



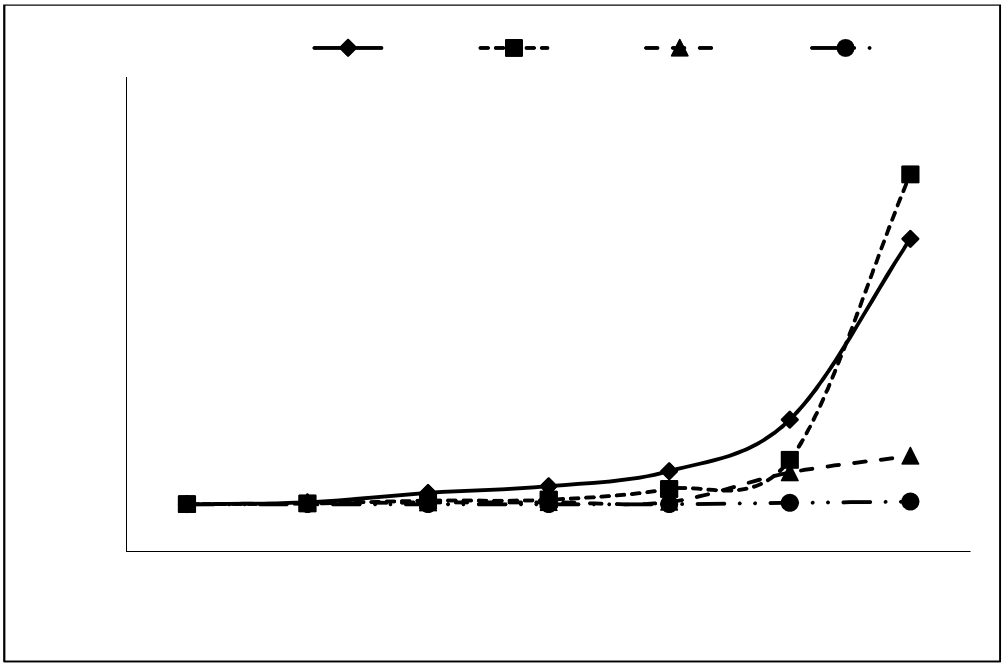


Fig. 1: Cu content in various plant parts of BL and 20SZ ecotypes and nutrient solution after 6 weeks experiment.

4.1.1.2. Bioconcentration and transportation factors of giant reed ecotypes

The bioconcentration (BCF) and transportation (TF) factors (TF) for both BL and 20SZ ecotypes showed high efficiency for concentrate and transport Cu from aqueous solution (Fig. 2a, b). For instance, BCF of BL and 20SZ ecotypes was above 1, where the highest estimated values for BL and 20SZ were 5.34 and 5.06, respectively, for Cu treatment of 1 and 26.8 mg L^{-1}, respectively. On the contrary, the lowest values were 2.93 and 0.76 at 5 and 1 mg L^{-1} treatment, respectively, for BL and 20SZ ecotypes, respectively.

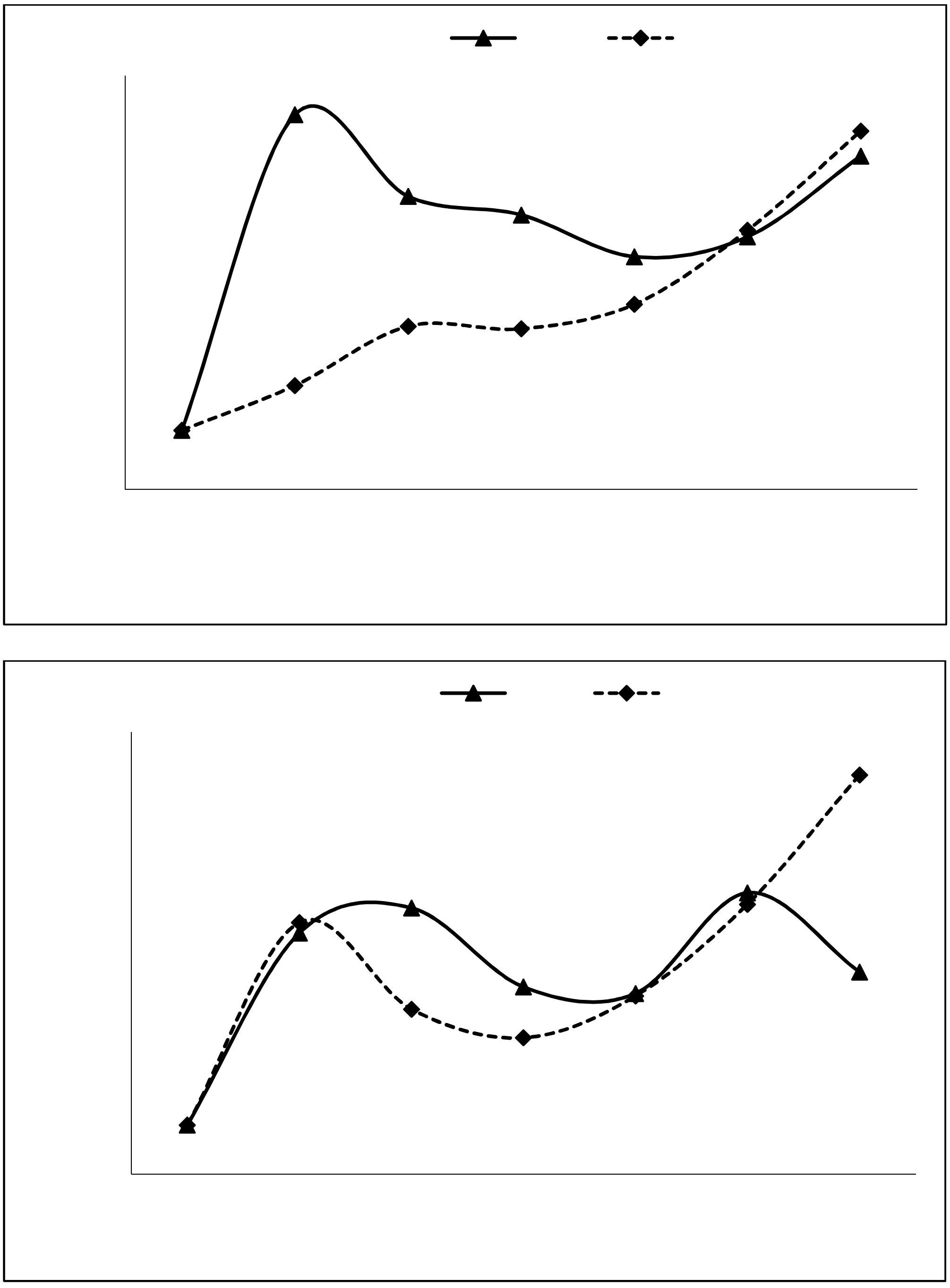


Fig. 2: **a**, Bioconcentration factor (BCF) of BL and 20SZ ecotypes at elevated Cu concentrations. **b**, Transportation factor (TF) of BL and 20SZ ecotypes at different Cu concentrations. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

However, estimated values of BCF linearly increased along with increasing Cu concentrations in growth medium for 20SZ ecotype, indicating that 20SZ ecotype significantly has considerable efficiency for Cu removal at high concentration in Cu-contaminated water bodies. But for BL ecotype, the BCF fluctuated with Cu concentration in culture medium. Moreover, the highest value 5.34 estimated at the lowest Cu treatment (1 mg L^{-1}) and along with increase Cu concentration the value of BCF decreased until 5 mg L^{-1} treatment and started to increase again recording 4.64 at 26.8 mg L^{-1} treatment. On the other hand, TF in both ecotypes was almost down 1 except for 20SZ at highest treatment (26.8 mg L^{-1}) since it was 1.43. The highest calculated values for TF were 0.95 and 1.43 at 10 and 26.8 mg L^{-1}, respectively, for BL and 20SZ ecotypes, respectively. Similarly, same results for TF of giant reed plants grown on Cu-contaminated soil were documented by Wu Qi et al. (2012), where they recorded that TF for giant reed plant was less than 1.

BCF of both ecotypes was above the reference value (1.0) for hyperaccumulation, and TF was almost around the reference values (1.0) and above this value in 20SZ ecotype at the highest Cu treatment. This might explain why we could not observe toxicity symptoms in the shoots of both ecotypes plants at that concentration. However, both TF and BCF increased at 26.8 mg L^{-1}, and plants showed some toxicity symptoms at that Cu treatment. These toxicity symptoms included appearance of short root and shoot length especially with BL ecotype, brown root tips, yellowing of leaves, and development of necrotic leaf margins.

4.1.1.3. Bioaccumulation percent and removal rate of Cu

Data clearly illustrated Cu was concentrated predominantly in roots (51.4–65.1 %) and culm (17.9–28.2 %) followed by leaves (15.7–24.4 %) for BL ecotype. While 20SZ ecotype was not so far from BL ecotype, where Cu was accumulated in roots (41.2–73.8 %) and culm (18.4–51.3 %) followed by leaves (7.5–19.7 %) (Table 1). In general, by looking at the average values for Cu bioaccumulation percent (BAP) in different plant parts of both ecotypes under different Cu treatment, it can be concluded that almost 59.3 % of Cu uptake by BL ecotype retained in its roots and 28.2 % in culm followed by 12.5 % in leaf blades. Whereas the accumulated Cu in plants of 20SZ ecotype was distributed as follow: 58.6 % in roots, 22.6 % in culm, and 18.9 % in leaf blades. Therefore, when giant reed is applied for phytoremediation purposes, it is important to take off totally the roots (rhizomes) from the growth medium and even soil or wastewater. From the mentioned data above, it becomes clear that both ecotypes were good candidates to treat Cu-contaminated water streams. It has been reviewed by Pei-ying et al. (2010) that 54–61 % of total removal Cu by *Hydrilla verticillata* plants grown on aqueous solution was in its root. Six weeks growth for both ecotypes was enough to treat and remove totally Cu from the growth medium successfully. Table 1 showed that the removal rate was the same for both ecotypes and ranged between 96.6 and 98.8 % at 2 and 26.8 mg L^{-1} treatments for BL ecotype. Total removal 100 % was observed with 20SZ ecotype at 1, 2, 3, and 5 mg L^{-1} treatments then slightly decreased with increase Cu concentration in culture medium achieving 97.0 and 98.0 % at 10 and 26.8 mg L^{-1} treatments, respectively.

Table 1: Effect of different Cu concentrations on removal rate (RR) and bioaccumulation percent (BAP) of BL and 20SZ ecotypes

Cu (mg L^{-1})	Bioaccumulation percent (BAP), %						Removal rate (RR), %	
	BL ecotype			20SZ ecotype			BL ecotype	20SZ ecotype
	Root	*Culm*	*Leaf*	*Root*	*Culm*	*Leaf*		
0	0.0g	0.0f	0.0f	0.0g	0.0f	0.0f	0.0g	0.0d
1	56.1d	28.2a	15.7e	54.8d	20.5de	24.8a	98.5b	100.0a
2	53.1e	22.5c	24.4a	68.0b	21.4d	10.6c	96.6f	100.0a
3	64.0b	17.9e	18.0d	73.8a	18.4e	7.8d	97.5d	100.0a
5	65.1a	19.6d	15.3e	65.6c	30.1b	4.4e	97.2e	100.0a
10	51.4f	27.6b	21.0b	52.7e	27.6c	19.7b	98.2c	97.0c
26.8	61.6c	19.5d	18.9c	41.2f	51.3a	7.5d	98.8a	98.0b

In the columns are different letters show significant differences among each group of treatments according to Duncan's test at $p < 0.05$

4.1.1.4. Cu sensitivity and plant growth

The concentration–response relationship (shoot biomass and culm length) for BL and 20SZ ecotypes exposed to increasing Cu concentrations in growth medium during the present investigation is depicted in Fig. 3. In general, results obtained showed that increasing Cu concentration in the nutrient solution slightly decreased the growth of both ecotypes. However, BL ecotype was more sensitive for increasing Cu concentration in culture medium than 20SZ ecotype. Where, gradually decreasing in shoot biomass percent compared to control plants was found. At 26.8 mg L^{-1} treatment for BL ecotype, 30 % reduction in shoot biomass was recorded compared to control plant. Otherwise, 20SZ ecotype plants showed more tolerant characters, where 45.7 % increasing in shoot biomass was found at 5 mg L^{-1} treatment, but more than 63 % reduction in shoot biomass was noticed at the highest treatment. The performance of culm length was similar to shoot biomass.

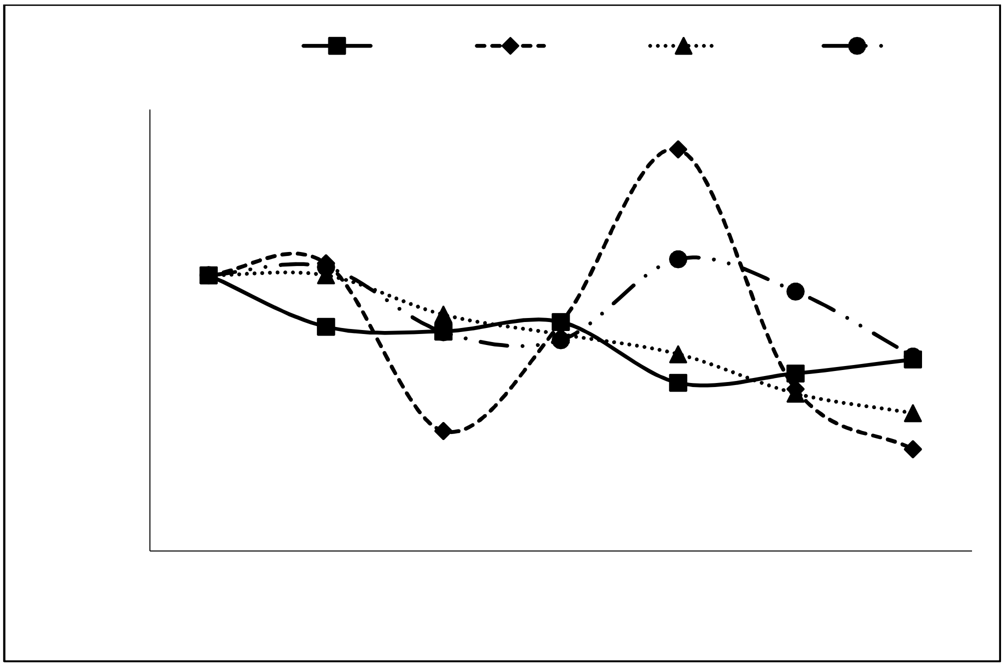


Fig. 3: Effect of Cu concentrations on shoot biomass and culm length of BL and 20SZ ecotypes of giant reed. Growth is expressed as a percentage of the no Cu control.

However, 1 mg L^{-1} treatment for both ecotypes induced culm length above the control plants achieving 100 and 102.9 % for BL and 20SZ, respectively, compared to control treatment. The length of culm was inhibited at the highest concentration of Cu (26.8 mgL^{-1}) by 50 and 29.4 % for BL and 20SZ ecotypes, respectively (Fig. 4).

Data of number of new buds of giant reed ecotypes investigated in current study is presented in Fig. 5. In general, no big differences for number of new buds of BL ecotype were found. Under all Cu treatments, the mother plant was able to regenerate 1.4 new buds per individual plant, whereas 20SZ plants were able to give 4.4 new buds per each mother plant at 5 mg L^{-1} treatment. From the mentioned data above, it could be clearly concluded that both ecotypes had capability to remove Cu totally from liquid medium achieving at the same time high biomass for energy production purposes.

Fig. 4: **a,** Plants of BL ecotype grown on different supplied Cu concentrations. **b,** Plants of 20SZ ecotype grown on different supplied Cu concentrations.

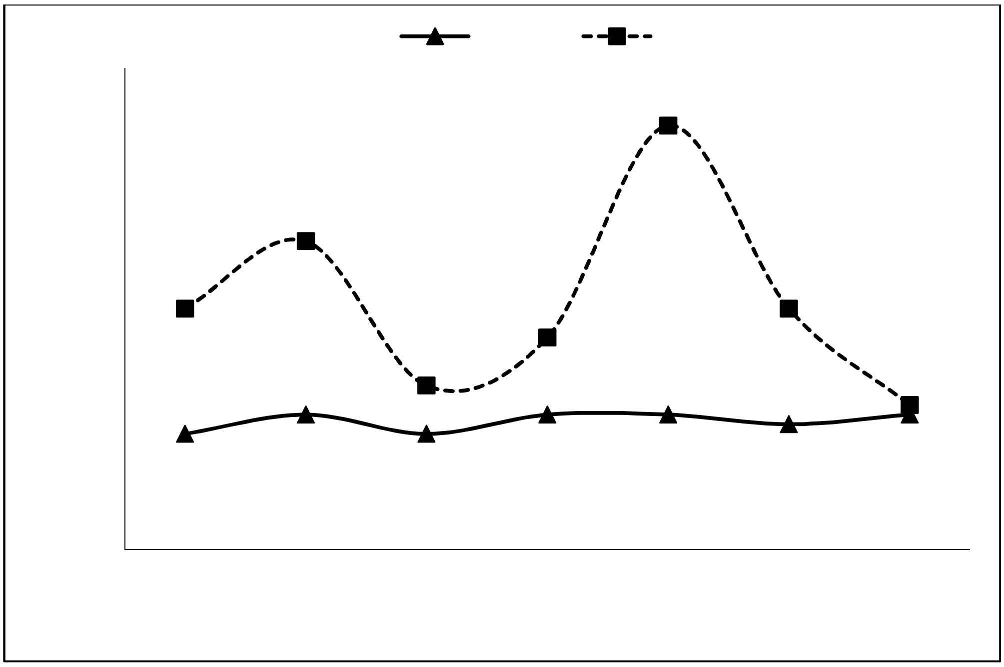


Fig. 5: Number of new buds of BL and 20SZ ecotypes at various applied Cu concentrations. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

Both somatic embryo-derived ecotypes of giant reed, BL (American ecotype) and 20SZ (Hungarian ecotype) accumulated large concentrations of Cu in their shoots (>48 and 79 mg kg^{-1} DW, respectively) when grown on hydroponic culture for 6 weeks in the solution with Cu concentration of 26.8 mg L^{-1} (Fig. 1). Both ecotypes showed high efficiency for Cu removal from wastewater bodies. As we have seen that BL and 20SZ ecotypes were able to tolerate Cu concentrations up to 10 mg L^{-1}, whereby no toxicity symptoms appeared in the leaves. The appearance of some toxicity symptoms in the leaves, short root length, brown root tips, and suppression of growth indicated that BL cannot tolerate Cu concentration above 26.8 mg L^{-1} while 20SZ ecotype can tolerate Cu-contaminated wastewater above 26.8 mg L^{-1} (Fig. 4). The bioaccumulation ability of Cu was much higher in 20SZ ecotype than BL ecotype where its values were above reference value 1 for both ecotypes. TF factor was similar to that estimated for BCF (Fig. 2), but its values were almost close to the reference value 1 for hyperaccumulators.

Thus, according to BCF and TF of both ecotypes, especially 20SZ ecotype is best classified as suitable to treat Cu-contaminated water bodies. Generally, giant reed is a nonfood crop that is not eaten by humans and is abundant near wetlands. Thus, its ability to accumulate metals in the stalk and leaves above the root concentration is a positive indicator of its potential capacity to serve as a phytoremediation plant linked with the nonexistence of a sign of the toxic effect of the metals on the plant up to Cu concentration of 26.8 mg L^{-1} in aqueous environments. Chlorosis and reduction of growth and root length are the most common symptoms of Cu toxicity in plants (Reichman, 2002). Chlorosis is frequently reported as an initial symptom and, with increased exposure, necrosis can appear in the leaf tips and margins. Both ecotypes showed no toxicity symptoms till 10 mg L^{-1} treatment (Fig. 4), only BL ecotype at 26.8 mg L^{-1} recorded short root and culm length with yellowish color of leaves.

Plants tend to accumulate Cu in their root tissues with small transportation to the shoots. Therefore, alterations in root growth and morphology occur before any toxic symptoms appear on shoots (Sheldon and Menzies, 2005). For both ecotypes BL and 20SZ, more than 59 and 58% of total Cu in plant tissues was found in root only (Table 1). Roots are especially vulnerable to Cu toxicity. Roots of *Origanum vulgare* exhibited severe reduction of length and root volume as well as generalized malformations after 2 months of growing in Cu-enriched soil (0.3–25.5 mg kg^{-1}). Higher plants have a set of mechanisms that help it tolerate high concentrations of different metals. The main aim of these mechanisms is to regulate the intracellular metal concentration, avoiding its excessive accumulation at metabolic sensitive sites within the cells and tissues. Reduction of metal uptake seems to be a primary line defense against metal accumulation and its consequent effects. Subsequently, cellular mechanisms of metal detoxification work to guarantee the fine regulation of Cu concentration inside different cells and tissues. At last, the enhancement of antioxidative defense plays an important role on the prevention of any damage caused by reactive oxygen species (ROS) occasionally produced when the homeostatic mechanisms are not enough to withstand the metal uptake (Clemens, 2001; Yruela, 2005). Giant reed showed little differences where it was able to uptake a significant amount of Cu from Cu-contaminated water. Therefore, giant reed maybe avoids the reduction of metal uptake, while it may have a different mechanism to tolerate high concentrations of Cu. The mechanism of Cu uptake, tolerance, and role of rhizospheric microbes needs further investigation with somatic embryo-derived ecotypes of giant reed.

4.1.2. Experiment 2: growing giant reed ecotypes on solidified medium

4.1.2.1. Cu toxicity symptoms

This experiment was carried out to investigate the impacts of Cu on growth of two ecotypes (BL and 20SZ) of giant reed plants in order to determine the possibility to use Cu-contaminated soils for biomass production during the phytoremediation processes of these soils. In general, neither ecotype (BL and 20SZ) showed any noticeable foliar symptoms as a result of Cu toxicity when they grew on a series of Cu concentrations up to 10 mg L^{-1}. In the treatment with 26.8 mg L^{-1} of Cu, shoots of BL plants showed higher susceptibility to the high Cu concentration than those of 20SZ. While 20SZ plants did not show any toxicity symptoms for shoot part, BL plants showed little difference in growth, i.e., burned edges of young leaves, twisted leaves, slight chlorosis and reduced growth were observed. In contrast, low Cu concentrations, i.e., 1, 2, 3 mg L^{-1} stimulated the growth of shoot parts of both BL and 20SZ (Fig. 6).

Adding Cu to the growth medium at increasing concentrations up to 5 mg L^{-1} positively affected the growth of root systems of BL and 20SZ plants as well. Well developed and healthy root systems were seen; as well as root hairs were present in both ecotypes. Additionally overall root morphology was investigated. In the lowest Cu concentrations, root systems were distinguished with fewer, longer roots but in the highest Cu concentrations, i.e., 10 and 26.8 mg L^{-1}, the plants possessed more and shorter roots (Fig. 6). In both BL and 20SZ, the highest Cu concentrations (10 and 26.8 mg L^{-1}) inhibited the growth of root systems. Roots were slightly stunted, and the root cuticle was thickened, and brown, especially for the BL ecotype. Neither lateral roots nor root hairs were clearly seen on the root systems of both ecotypes. The previously mentioned symptoms of Cu toxicity on the root system were clearly observed in BL plants compared to 20SZ plants which seemed to be more tolerant to high Cu concentrations (Fig. 6).

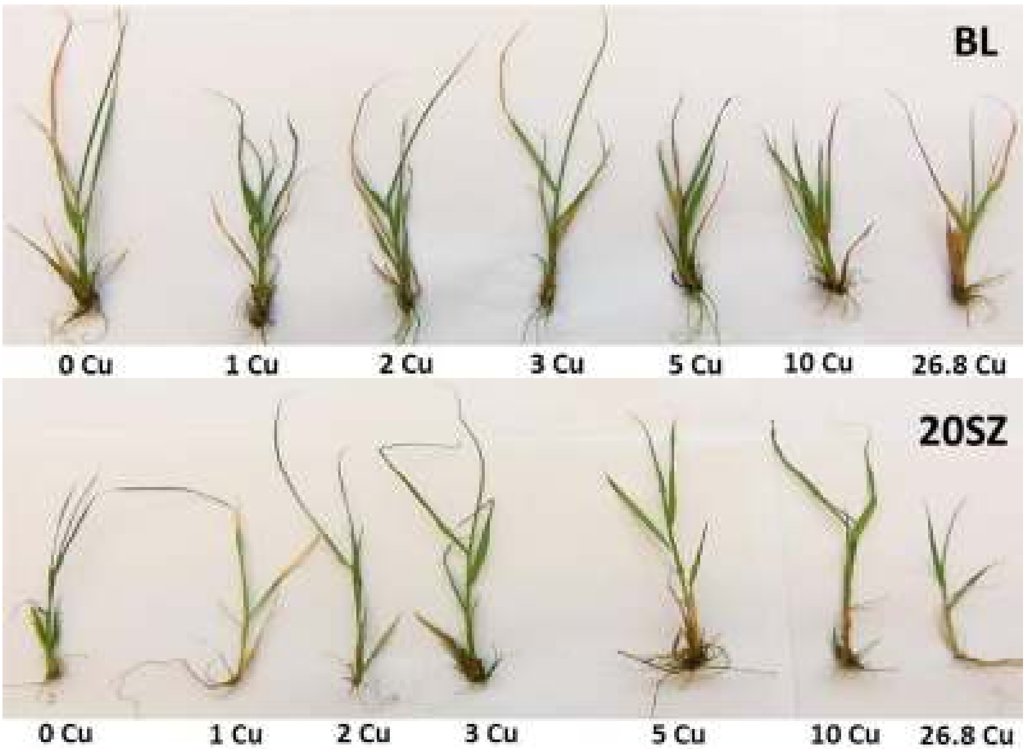


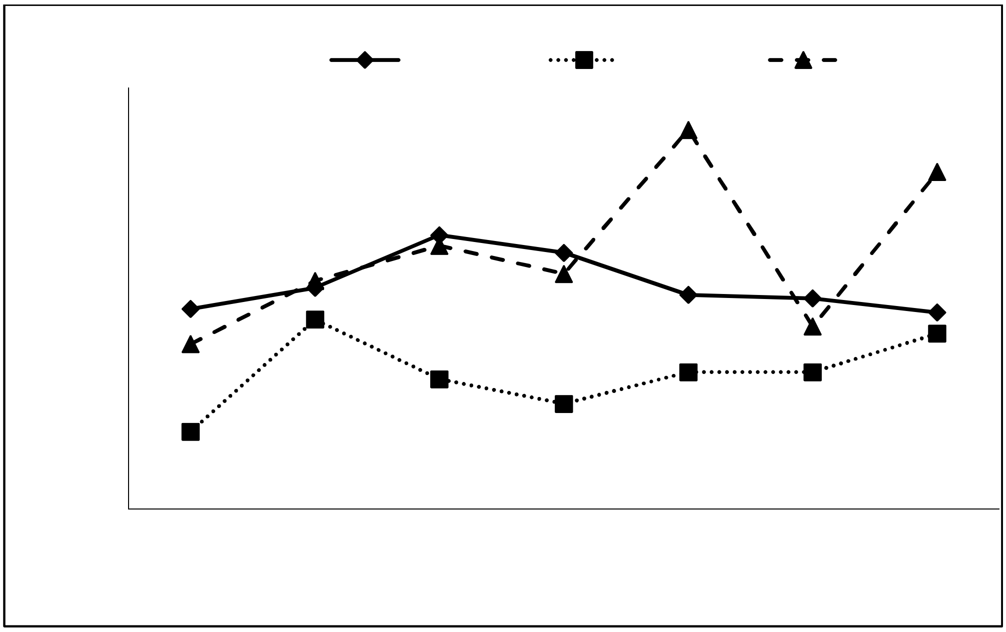
Fig. 6: Cu toxicity symptoms on BL and 20SZ plants after growing 6 weeks on a series of elevated Cu exposures.

4.1.2.2. Plant biomass yields

Plant biomass can be considered as a good indicator for the overall health of a plant growing in the presence of potential toxic metals such as Cu. At the end of the experiment, i.e., six weeks from transplantation, vegetative parameters of plants were recorded in order to assess the effects of a series of Cu doses on plant growth parameters. Growth of both BL and 20SZ ecotypes of giant reed in reference to fresh, and dry mass of roots, culms, leaves and plants are depicted in Fig. 7a, 7b, 8a, and 8b. The lowest Cu concentrations (1, 2, 3, 5, 10 mg L^{-1}) in growth medium had a positive effect on the growth of BL plants resulting in increasing fresh mass of the roots by 4 to 31% compared to control. However, the highest increase of fresh mass of BL roots 31% was measured at treatment of 2 mg L^{-1} of Cu in growth medium (Fig. 7a). In contrast, the highest Cu concentration (26.8 mg L^{-1}) insignificantly reduced the fresh mass of BL roots by 1%. On the other hand, different results were recorded for fresh mass of roots of 20SZ ecotype plants. Furthermore, adding Cu to the growth medium at either 1 or 3 mg L^{-1} decreased the fresh mass of the roots by 2%. Nevertheless, adding 26.8 mg L^{-1} of Cu to the 20SZ plants enhanced the fresh mass of roots by 16% compared to control treatment. However, 2 mg L^{-1} of Cu increased significantly the fresh mass of roots, achieving the highest increase for fresh mass of roots among the other treatments by 129% (Fig. 7b).

Plant culm is the main portion of total biomass of giant reed. Therefore, it is important to precisely analyse the effect of Cu on culm biomass. Fresh mass of plant culm of BL and 20SZ ecotypes is represented in Fig. 7a and 7b. Cu doses significantly increased the fresh mass of culm for BL and 20SZ plants compared to control. In BL plants, while the highest increase in fresh mass of culm (100%) was found at the 1 mg L^{-1} Cu level the lowest increase (25%) compared to control (no Cu) was noticed at 3 mg L^{-1} of Cu. However, treatment 26.8 mg L^{-1} Cu achieved an increase of some 88% compared to no Cu addition. On the other hand, for 20SZ plants, treatment with 2 mg L^{-1} encouraged the plant growth where it increased the fresh mass by 112%. A 65% increase of fresh mass of plant culm, as a lowest increase compared to control, was measured when Cu concentration in growth medium was 26.8 mg L^{-1}.

Impacts of a series of Cu concentrations on fresh and dry masses of leaf blade of both BL and 20SZ ecotypes are displayed in Fig. 7a, 7b, 8a, and 8b. Leaf blade of BL plants was more tolerant to high Cu concentrations than leaf blade of 20SZ plants. However, a significant increase in fresh mass of leaf blade of BL plants was recorded in all treatments compared to control. Moreover, the highest Cu concentration in growth medium caused the leaf growth to increase by 86%. But the treatment of 5 mg L^{-1} of Cu increased the fresh mass by 107%, the highest measured increase. In contrast, leaf blades of 20SZ plants were less tolerant to high Cu doses than leaf blades of BL plants; treatments of 3, 10, and 26.8 mg L^{-1} of Cu gradually declined the fresh mass of leaf blades by 2, 26, and 35% respectively. However, adding 5 mg L^{-1} of Cu slightly increased the fresh mass by 9%, treatment by 2 mg L^{-1} of Cu achieved the highest increase in fresh mass by 81% compared to untreated plants.



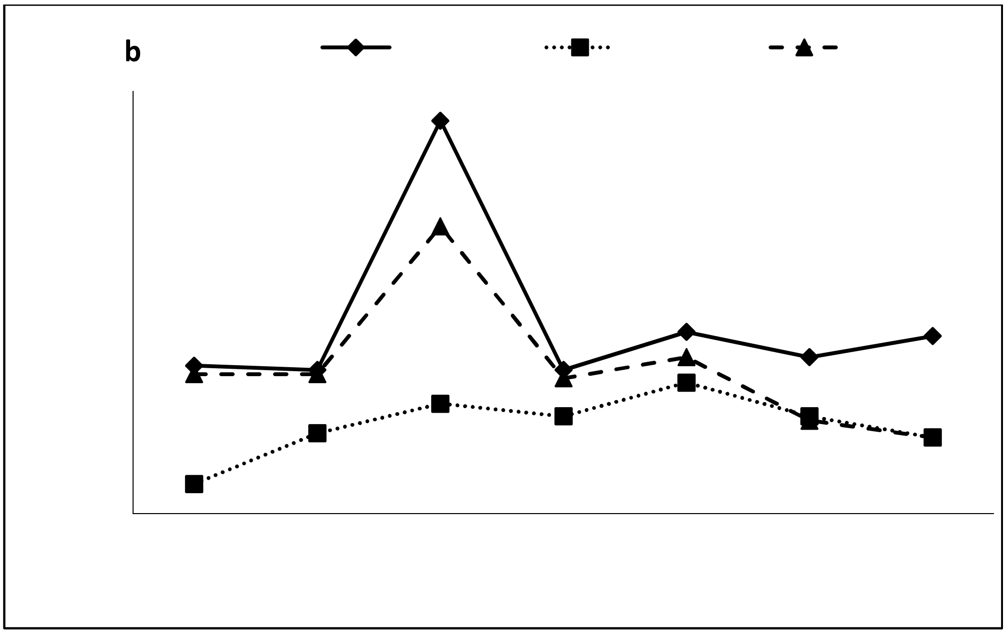


Fig. 7: Fresh mass of different plant parts of giant reed plant growing on a series of Cu concentrations. (a) BL ecotype. (b) 20SZ ecotype. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

Data of dry mass of roots of BL ecotype plants showed significant differences between treatments. In contrast with values of fresh mass of roots, the values of dry mass of roots showed positive effects of increasing supplied Cu concentrations. Dry mass of roots positively correlated with increasing Cu doses in growth medium (r = 0.65). Unexpectedly, the highest supplied Cu level caused the largest increase of dry mass of BL roots, almost 78% increase compared to control (Fig. 8a). In general, all Cu treatments increased the dry mass of BL roots compared to control. Likewise, in 20SZ ecotype, the highest increase, 114%, of dry mass of 20SZ roots also was found at the highest Cu level (26.8 mg L^{-1}). Moreover, all supplied Cu doses increased the dry mass of 20SZ roots (Fig. 8b). Also, data of dry mass of roots showed a positive correlation with Cu concentrations in growth medium (r = 0.68). Similarly, the findings of 20SZ ecotype were same as in BL ecotype.

All Cu treatments increased the dry mass of plant culm of both BL and 20SZ ecotypes significantly (p < 0.05) compared to control (Fig. 8a and 8b). However, dry mass of BL culm recorded the highest increase by 367% at the highest supplied Cu in growth medium (26.8 mg L^{-1}) while treatment 3 mg L^{-1} exhibited the lowest increase (67%) in dry mass of BL culm. Data showed a positive correlation (r = 0.70) between Cu treatments and dry mass of BL culm. About dry mass of 20SZ culm, treatment 5 mg L^{-1} achieved the highest increase in dry mass by 400%. While, the highest Cu treatment (26.8 mg L^{-1}) increased the dry mass by 200%, 3 mg L^{-1} of Cu displayed the lowest increase in dry mass (100%).

Significant differences were found between treatments for dry mass of leaf blades of the two ecotypes (BL and 20SZ). In BL plants, the highest increase, 94%, in dry mass was obtained at the highest Cu treatment (26.8 mg L^{-1}). Moreover, dry mass of leaf blades of treated plants with 10 mg L^{-1} of Cu increased by 13%, while 1 mg L^{-1} of Cu in growth medium was not enough to improve the growth, therefore the dry mass of leaf blades at this concentration decreased by 6%. Dry mass of leaf blades of 20SZ plants changed with supplied Cu concentrations significantly (p < 0.05), where Cu doses up to 5 mg L^{-1} increased the dry mass of leaf blades by 11-133% at 3 and 2 mg L^{-1} of Cu, respectively. At 10 mg Cu $L-^{1}$, 22% reduction in leaf blade dry mass of 20SZ plants was noticed, while no reduction in leaf blade dry mass was reported at 26.8 mg Cu L^{-1}.

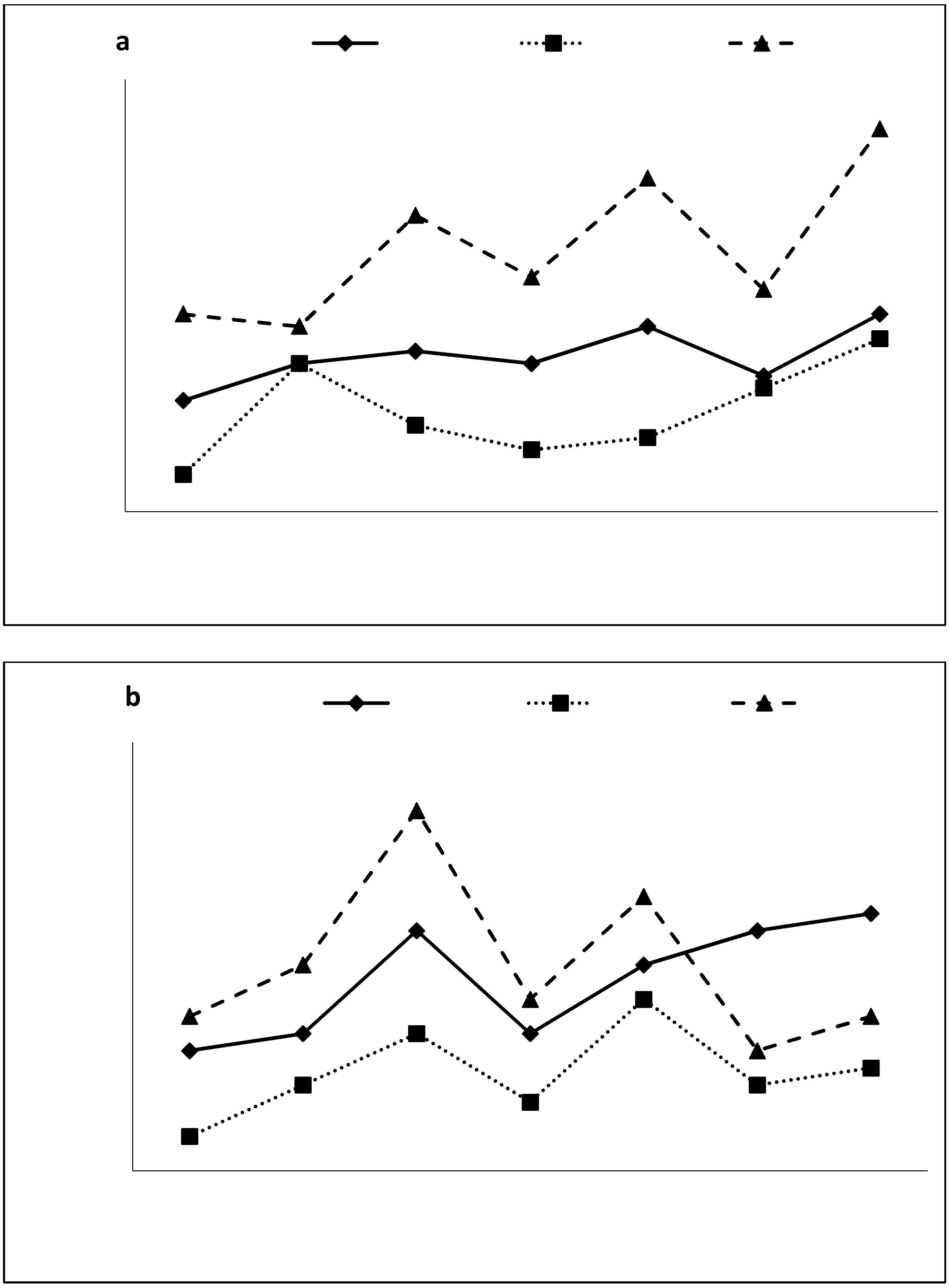


Fig. 8: Dry mass of different plant parts of giant reed plant growing on increasing Cu concentrations. (a) BL ecotype. (b) 20SZ ecotype. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

Nevertheless, it could be concluded that increasing Cu concentrations, in general, did not show any adverse effects on plant biomass. All Cu treatments increased the fresh and dry mass of plants of BL and 20SZ ecotypes, where the highest increase in fresh mass of whole plant were 53 and 107% for BL and 20SZ ecotypes, respectively, at treatments of 5 and 2 mg L^{-1} of Cu, respectively. Furthermore, the dry mass of BL and 20SZ plants recorded the highest increases 118 and 139%, respectively, at Cu concentrations of 26.8 and 2 mg L^{-1}, respectively (Fig. 7a, 7b, 8a, and 8b). Additionally, the highest Cu concentration in growth medium did not show adverse effects on plant fresh and dry mass in either ecotype compared to untreated plants.

4.1.2.3. Growth rate of giant reed

Figure 9 displays the length of shoot and root of both BL and 20SZ plants. In BL plants, shoot and root length, respectively, negatively correlated with increasing Cu treatments ($r_{(shoot)}$ = -0.60 *and* $r_{(root)}$ = -0.70). However, high Cu concentrations (10 and 26.8 mg L^{-1}) had adverse effects on shoot and root length, where shoot length significantly decreased by 10 % at both Cu concentrations, while root length was reduced by 13, and 38% at treatments of 10 and 26.8 mg L^{-1} of Cu, respectively. Cu at 5 mg L^{-1} did not affect the shoot length; it reduced the root length by 25% compared to control plants. However, the low Cu concentrations, i.e., 1, 2, and 3 mg L^{-1}, increased the shoot length by 30-40% and root length by 13-50%, where the largest increases were noticed at treatment of 1 mg L^{-1} of Cu. On the other hand, 20SZ plants showed higher tolerance to Cu toxicity; where all treatments of Cu increased the shoot length significantly except the highest Cu treatment (26.8 mg L^{-1}) which reduced the length of shoot by 43%. This adverse effect of most elevated Cu treatment had not been recorded in root length. Moreover, treatment of 26.8 mg L^{-1} of Cu increased the root length by 67% compared to untreated plant root. Neither treatment 3 nor 10 mg L^{-1} of Cu affected root length, but 100% increase in root length was found under treatment of 1 mg L^{-1} of Cu.

Number of new buds per plant is presented in Fig. 10. Number of new buds was significantly affected by increasing Cu treatments. However, in BL ecotype, treatments of 1, 2, 3, and 5 mg L^{-1} of Cu increased the number of new buds by 8-23%. Though treatment of 10 mg L^{-1} did not affect the number of new buds, treatment of 26.8 mg L^{-1} decreased the number of new buds by 8% compared to control.

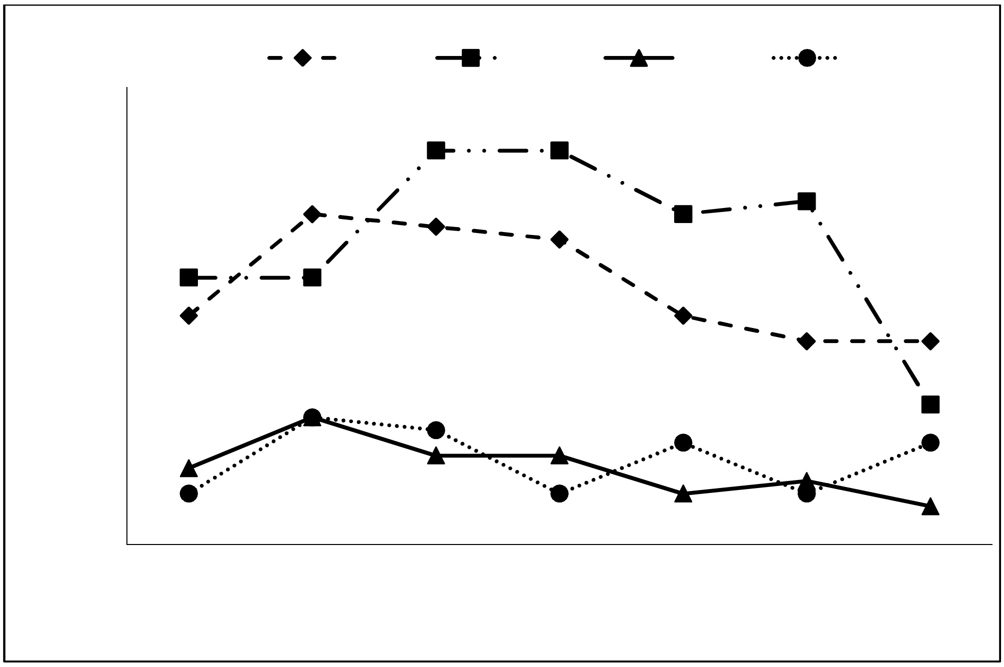


Fig. 9: Effect of elevated Cu concentrations on shoots and root length of two ecotypes (BL and 20SZ) of giant reed. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

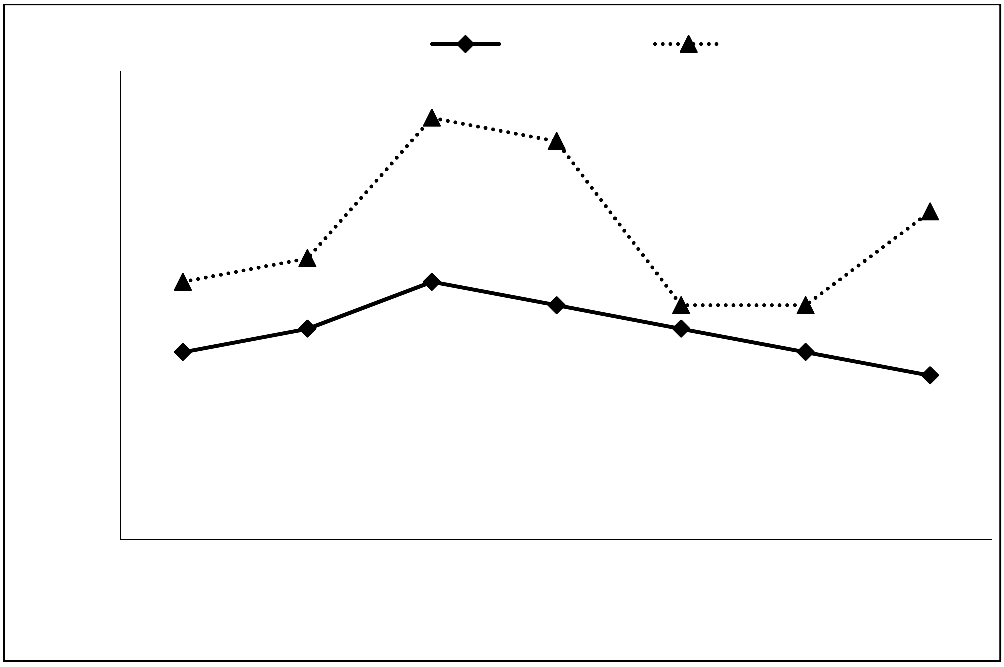


Fig. 10: Effect of increasing Cu concentrations on number of new buds of two ecotypes (BL and 20SZ) of giant reed. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

4.1.2.4. Cu uptake

The capacity of giant reed ecotypes (BL and 20SZ) to uptake Cu from a growth medium supplied with increasing doses of Cu is shown in Table 2. There were significant differences between Cu treatments and Cu content in plant organs. Moreover, data showed that Cu contents in plant parts of both ecotypes, i.e., root, culm and leaf, were positively correlated with Cu in growth medium. Generally, BL and 20SZ ecotypes had the same behaviour towards Cu uptake within low levels up to 5 mg L^{-1}, where gradual increase in Cu content through plant parts was found in both ecotypes. In addition, in BL plants, the highest Cu content was found in the root, followed by the culm, and the lowest Cu content was recorded in leaf. But in 20SZ plants Cu content was higher in leaf than culm; however roots of 20SZ accumulated more Cu than culm and leaf. With increasing Cu in growth medium the response of plants to Cu changed, where Cu content in the root of BL plants dramatically increased by almost four times in the treatment of 10 mg L^{-1} compared to its Cu content in the treatment of 5 mg L^{-1}. On the one hand, treatment by 26.8 mg L^{-1} also caused an increase in Cu content in BL root by almost 8x compared to treatment of 5 mg L^{-1}. Likewise, 20SZ root also showed the same tendency in treatment by 26.8 mg L^{-1} of Cu showing some of three times increase in Cu content compared to treatment by 5 mg L^{-1} (Table 2). However, Cu content of the culm of BL plants linearly increased with increasing Cu doses up to 10 mg L^{-1} to 3.34 µg g^{-1} DM. While the highest Cu treatment resulted in almost 4 folds increase in Cu content in culm of BL plants, such increase was not found in the culm of 20SZ plants compared to treatment of 10 mg L^{-1}, where gradual increase of Cu content only was seen in culm of 20SZ plants (Table 2). In BL leaf, the Cu uptake had linear relation to the increasing supplied Cu concentrations. Leaf Cu content at various treatments significantly ($p < 0.05$) differed. However, increasing Cu level had an incremental effect on leaf Cu content, where at treatment of 3 mg L^{-1} of Cu in growth medium leaf Cu content was 1.61 µg g^{-1} DM. But with increasing Cu level in growth medium the leaf Cu content increased drastically to 3.21, 9.07, and 27.31 µg g^{-1} DM at treatments of 5, 10, and 26.8 mg L^{-1}, respectively. However, leaf Cu content was higher in BL plant than 20SZ plant, where the highest Cu treatment resulted in 4.45 µg g^{-1} DM of Cu in leaf of 20SZ plant (Table 2).

Table 2: Cu content ($\mu g\ g^{-1}$ DM) in different plant parts of giant reed ecotypes (BL and 20SZ) after 6 weeks growing on elevated Cu concentrations

	BL ecotype				20SZ ecotype			
Cu (mg L^{-1})	*Root*	*Culm*	*Leaf blade*	*Medium*	*Root*	*Culm*	*Leaf blade*	*Medium*
0	0.00 g	0.00 e	0.00 g	0.00 g	0.00 f	0.00 g	0.00 e	0.00 e
1	2.07 f	1.44 d	1.08 f	0.01 f	2.21 e	0.66 f	1.14 d	0.00 de
2	2.30 e	1.46 d	1.35 e	0.01 e	2.01 e	0.91 e	1.16 d	0.00 d
3	3.21 d	2.72 c	1.61 d	0.01 d	3.09 d	1.52 d	1.88 c	0.00 d
5	5.49 c	2.73 c	3.21 c	0.02 c	5.90 c	2.22 c	1.97 c	0.01 c
10	18.53 b	3.34 b	9.07 b	0.04 b	6.84 b	4.63 b	4.74 a	0.03 b
26.8	39.23 a	13.06 a	27.31 a	0.11 a	15.70 a	4.98 a	4.45 b	0.08 a

Different letters in same column show significant differences among each group of treatments according to Duncan's test at $p < 0.05$

4.1.2.5. Bioaccumulation of Cu by giant reed

Data of bioaccumulation percent (BAP) of Cu within BL and 20SZ ecotypes is presented in Table 3. Results of data analysis showed significant differences between Cu treatments and Cu bioaccumulation percent throughout various plant organs in both ecotypes. In general, in BL plants, approximately 48.3% of total Cu content in plant was accumulated in the root while the culm of plant accumulated 24.5%. But the leaf showed greater ability to accumulate Cu, where it accumulated 27.2% of Cu. In particular, in case of high Cu treatments, i.e., 10 and 26.8 mg L^{-1}, plant root of BL ecotype accumulated more Cu compared to low Cu treatments. The percentages of Cu bioaccumulation were 59.9 and 49.3% at treatments of 10 and 26.8 mg L^{-1}, respectively. Similar results were obtained in the leaf blade, where the higher percentages of Cu bioaccumulation, i.e., 29.3 and 34.3%, occurred at treatments of 10 and 26.8 mg L^{-1}, respectively. In contrast, the culm showed different behaviour towards Cu bioaccumulation under high Cu treatments. The lowest percentages of Cu bioaccumulation, i.e., 10.8 and 16.4%, were observed at treatments of 10 and 26.8 mg L^{-1}, respectively, compared to low Cu concentrations up to 5 mg L^{-1}. Likewise, the same tendency was found in 20SZ plants, 52.6, 22.1, and 25.3% in root, culm and leaf blade, respectively. However, the response of 20SZ plant to high Cu concentrations in growth medium was entirely different from that of BL plant. In the root of 20SZ ecotype, the highest percentage of Cu bioaccumulation was 62.5% at the highest Cu treatments, while treatment of 10 mg L^{-1} of Cu resulted in the highest percentages of Cu bioaccumulation in culm and leaf blade by 28.6 and 29.2%, respectively (Table 3). Additionally, in the highest Cu treatment both culm and leaf blade of 20SZ plant showed low capacity to accumulate Cu compared to low Cu treatments.

Table 3: Cu-bioaccumulation percent of BL and 20SZ ecotypes of giant reed plant after growing on different Cu concentrations

	BL ecotype			20SZ ecotype		
Cu (mg L^{-1})	*Root*	*Culm*	*Leaf blade*	*Root*	*Culm*	*Leaf blade*
0	0.0 e	0.0 g	0.0 g	0.0 f	0.0 e	0.0 f
1	45.2 c	31.3 b	23.5 e	55.1 c	16.5 d	28.4 c
2	45.1 c	28.5 c	26.5 d	49.4 d	22.3 b	28.4 c
3	42.5 d	36.1 a	21.4 f	47.6 d	23.4 b	29.0 b
5	48.0 b	23.9 d	28.0 c	58.5 b	22.0 b	19.5 d
10	59.9 a	10.8 f	29.3 b	42.2 e	28.6 a	29.2 a
26.8	49.3 b	16.4 e	34.3 a	62.5 a	19.8 c	17.7 e

Different letters in same column show significant differences among each group of treatments according to Duncan's test at $p < 0.05$

4.1.2.6. Bioconcentration and transportation factors

The ability of BL and 20SZ ecotypes of giant reed to bioconcentrate and transport Cu from their roots to shoots when they were growing on a series of Cu concentrations is depicted in Fig. 11 and 12. At low Cu doses, both ecotypes had the same tendency towards bioconcentration of Cu; the highest calculated values of bioconcentration factor (BCF) in BL and 20SZ plants were 4.6 and 4.0, respectively, when Cu concentration in growth medium was 1 mg L^{-1}. Despite there being no definite correlation between Cu concentrations and BCF in either ecotype, significant differences were found between Cu treatments in both ecotypes. However, at low Cu doses both ecotypes had almost same values of BCF, but with increasing Cu concentration in growth medium BL plants showed higher values of BCF than 20SZ plants. At treatment of 26.8 mg L^{-1} of Cu, BCF values were 3.0 and 0.9 for BL and

20SZ plants, respectively (Fig. 11). Nevertheless, Cu treatments significantly affected values of TF in BL and 20SZ ecotypes. In whatever way, BL plants had values of TF greater than 1, i.e., a reference value, in all treatments except at 10 mg L^{-1} of Cu where TF was 0.7. Whilst, the highest TF value 1.4 was obtained at 3 mg L^{-1}, the highest Cu treatment, i.e., 26.8 mg L^{-1}, resulted in a TF value of 1.0. On the other hand, 20SZ plants had TF values lower than 1 at 1, 5, and 26.8 mg Cu L^{-1}, but the greatest TF value was 1.4 and was observed at 10 mg Cu L^{-1} (Fig. 12).

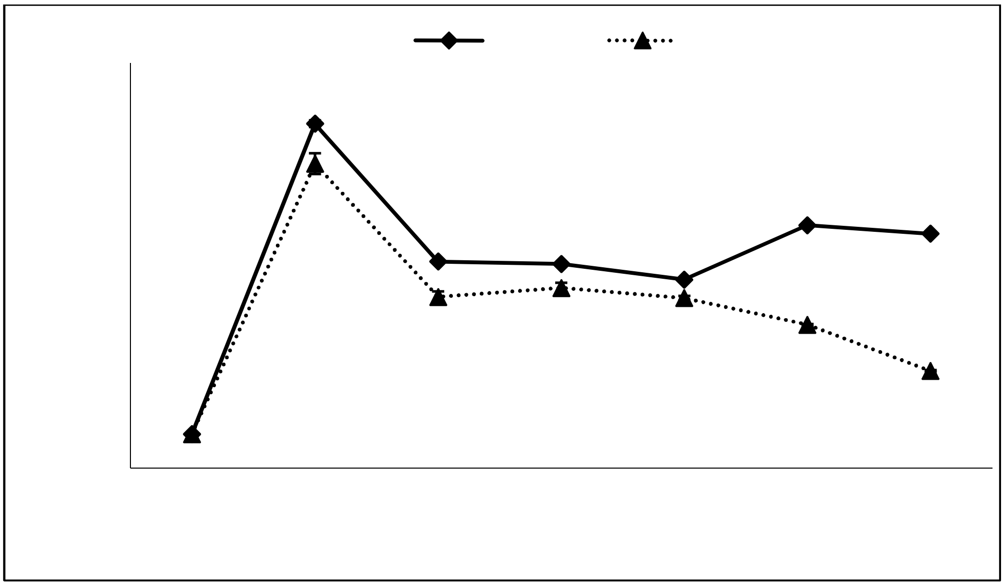


Fig. 11: Bioconcentration factor (BCF) of BL and 20SZ ecotypes of giant reed after growing on a series of Cu for 6 weeks. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

In the current investigation, BL and 20SZ ecotypes of giant reed were able to grow on elevated concentrations of Cu without any apparent foliar symptoms of Cu toxicity up to 10 mg L^{-1} of Cu. Basically, roots are the most sensitive part of plant to Cu toxicity. BL and 20SZ plants had healthy and well developed roots at Cu concentrations lower than 10 mg L^{-1}. However, these findings were different than those of Sheldon and Menzies (2005) who reported low Cu concentrations between 0.2 and 1 µM significantly reduced root growth and disrupted the root cuticle of Rhodes grass. Despite high Cu contents measured in shoots of BL and 20SZ plants, biomass of both ecotypes was significantly ($p < 0.05$) enhanced, where at 26.8 mg Cu L^{-1}, plants of BL and 20SZ recorded higher biomass than control plants.

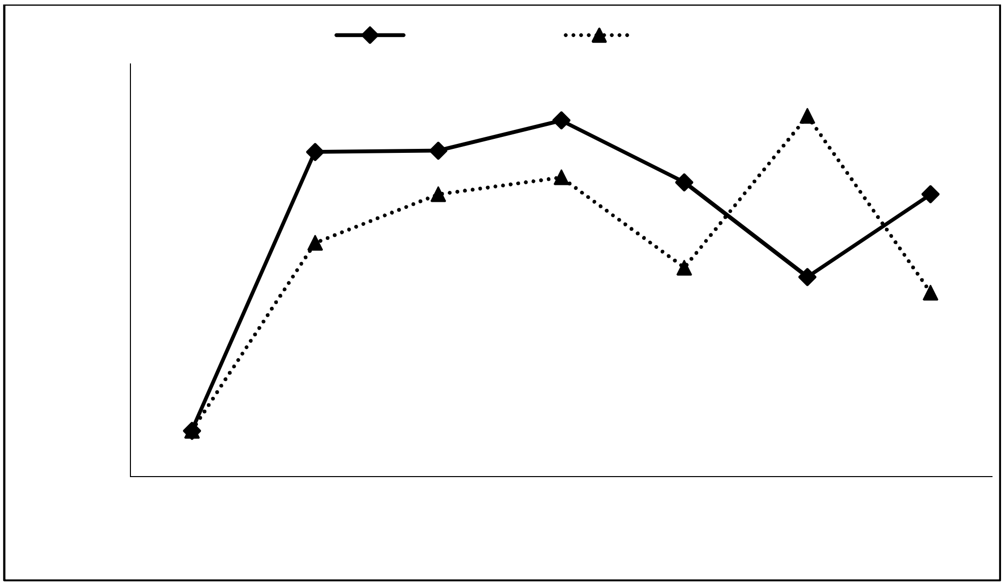


Fig. 12: Transportation factor (TF) of BL and 20SZ ecotypes of giant reed after growing on a series of Cu for 6 weeks. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

Content of Cu in plant tissues mainly depends on its concentration in growth medium (Mavrogianopoulos et al. 2002). Cu levels in growth medium up to 5 mg L^{-1} caused a gradual increase in Cu content in plant tissues of BL and 20SZ plants. In higher Cu treatments, BL plants had a different behavior than 20SZ, where Cu content in BL plant tissues increased exponentially. In the current study, root systems accumulated more Cu compared to culm and leaf blades in both ecotypes. Almost 50 % of total Cu content in plant tissues was found in root systems of BL, but root systems of 20SZ plants were able to accumulate higher Cu content. Mavrogianopoulos et al. (2002) reported Cu concentration in giant reed plants grown in a closed hydroponic system was 14 % compared to the plants grown in the soil.

Although, BL plants had higher Cu content in whole plant tissues compared to 20SZ plants, similar tendencies toward Cu accumulation in different plant tissues was found in both ecotypes. The average Cu accumulation (%) of all treatments in different plant parts of BL and 20SZ plants was in the following order: root > leaf blade > culm. The average Cu accumulation (%) was 48.3, 27.2 and 24.5 in root, leaf blade and culm of BL plants and 52.6, 25.3 and 22.1 of 20SZ plants, respectively. High Cu content in roots compared to shoots could be explained as giant reed plants accumulate Cu in their roots to a certain point and then transport it to shoots after roots become saturated (Mavrogianopoulos et al. 2002). Mavrogianopoulos et al. (2002) cited that giant reed populations (Naxos and Hania) accumulated Cu by 7 % and 22 % in leaves and roots respectively, when they grew in a closed hydroponic system. While Baker and Brooks (1989) reported similar tendencies for Cu in different plants, Hasselgren (1999) reported different results for Cu in willow, where the stem accumulated more Cu than roots. This might indicate the speciation of Cu within plant parts strongly depends on the plant species.

At 26.8 mg Cu L^{-1}, Cu content in leaf of BL plants was 27.31 µg g^{-1} DM, and this was higher than that found in leaf of 20SZ plants (4.45 µg g^{-1} DM). Apparently, this high level of Cu content in BL leaves is above the threshold of toxicity, since BL plants suffered burned edges of young leaves, twisted leaves, leaf discoloration and retardation of plant growth. This threshold should be between 4.45 and 27.31 µg g^{-1} DM, because 20SZ plants did not show any of these symptoms. Further investigation of a Cu threshold value for giant reed is warranted. Cu content in tissues of 20SZ plants was lower than that measured in BL plants, with 52.6 % of total Cu found in root systems. BL plants accumulated 48.3 % of total Cu in its root system. These findings might illustrate each ecotype has its own mechanism to tolerate high Cu concentrations in growth medium. Whereas BL plants prefer to uptake and accumulate more Cu in its tissues, 20SZ plants tolerate high Cu concentrations by avoiding uptake of Cu. The reason could be that tissues of 20SZ plants are sensitive to Cu moreso than those of BL plants that can stay healthy at high Cu contents in their tissues.

Bioconcentration factor (BCF) is the concentration of metal in plant tissues per concentration of same metal in growth medium. Metal hyperaccumulator plants are distinguished by a BCF higher than 1 (Mirza et al. 2011). In the current experiment, BL and 20SZ plants had BCF >1, meaning both ecotypes could be employed for Cu phytoremediation purposes. Alongside BCF >1, high biomass production of giant reed plants under unfavorable growth conditions is an additional advantage that emphasizes the benefits of using these plants for phytoremediation of Cu contaminated sites.

4.2. *Ex vitro* experiment at greenhouse

4.2.1. Vegetative parameters at harvest

At the harvest of experiment number of mother plant – which started the experiment – new tillers, length of plant, number of leaves, and length of internodes of STM, BL, and ESP ecotypes of giant reed were recorded (Tables, 4, 5, 6, and 7).

The pot experiment under greenhouse conditions – from July to October 2013 – had been started with 2 seedlings per pot of giant reed ecotypes. With aim to study the effect of Cu on the survival of plants, number of surviving mother plants is recorded at the end of experiment period. All ecotypes showed considerable tolerance for high Cu concentrations, where elevated Cu levels insignificantly (p < 0.05) influenced the number of mother plants which started the experiment. However, 2 mother plants per pot were recorded for STM ecotype at 200 mg Cu Kg^{-1}, while treatment of 400 mg Cu kg^{-1} recorded 1.8 mother plants for BL ecotype. For ESP ecotype, 1.8 mother plants per pot were found at Cu concentration of 300 mg kg^{-1} (Table 4). These results showed that giant reed ecotypes are able to grow on Cu-contaminated soil-like growth medium up to 400 mg kg^{-1} with survival percentage ranged from 90 to 100%.

Table 4: Number of mother plant and new tillers of giant reed ecotypes at harvest

	Mother plant			New tillers		
Cu (mg kg^{-1})	*STM*	*BL*	*ESP*	*STM*	*BL*	*ESP*
0	1.4ab	1.3ab	1.8ab	4.2a	4.0a	4.8a
100	2.0a	1.8ab	1.6ab	4.6a	6.0a	4.0a
200	2.0a	1.6ab	1.2b	6.0a	4.6a	4.4a
300	1.6ab	1.5ab	1.8ab	4.8a	6.0a	3.8a
400	1.8ab	1.8ab	1.6ab	4.2a	4.8a	3.6a

Different letters in same column show significant differences among each group of treatments according to Duncan's test at p < 0.05

On the other hand, number of new tillers per plant was insignificantly (p < 0.05) affected by increasing Cu doses especially for STM and BL ecotypes. While, 6 new tillers per plant were recorded under treatment of 200 and 300 mg kg^{-1} for STM and BL ecotypes, respectively, 4.8 new tillers were noticed for ESP ecotype at no supplied Cu. However, at highest Cu dose the data of new tillers was almost same with control treatment (Table 4). Therefore, giant reed could be recommended as phytoremdiation candidate as a result for its high survival percentage on Cu-contaminated soil-like growth medium which is among key characteristics that any proposed plant for phytoremediation purposes has to possess it (Fig. 13).

Spanish (ESP) ecotype showed positive response for increasing Cu concentrations in soil-like growth medium, where length of mother plant significantly ($p < 0.05$) increased from 262 to 291 mm when Cu was 0 and 400 mg kg^{-1}, respectively. Together, STM and BL ecotypes recorded similar results for mother plant length, where length of mother plant insignificantly reduced with increase Cu doses compared to control (Table 5). However, new tillers of all three ecotypes had same tendency towards elevated Cu levels. Length of new tillers was significantly increased with increasing Cu for all ecotypes compared to control. The tallest tiller of STM, BL and ESP was 353, 235, 343 mm at treatments of 100, 200, and 100 mg Cu kg^{-1}, respectively (Table 5). Short length of American (BL) ecotype could be genetically explained because even in control treatment, BL plants also were the shortest (Fig. 13).

Table 5: Length of mother plant and new tillers of giant reed ecotypes at harvest

	Mother plant			New tillers		
Cu (mg kg^{-1})	*STM*	*BL*	*ESP*	*STM*	*BL*	*ESP*
0	285ab	253ab	262ab	192e	185e	312abc
100	223ab	221ab	243ab	353a	214de	343ab
200	252ab	262ab	279ab	256bcde	235cde	285abcd
300	277ab	200b	296a	237cde	177e	247cde
400	273ab	221ab	291a	298abcd	181e	316abc

Different letters in same column show significant differences among each group of treatments according to Duncan's test at $p < 0.05$

Number of leaves of mother plants and new tillers at the end of experiment is presented in Table 6. Hungarian (STM) ecotype mother plants showed significant ($p < 0.05$) increase for number of leaves with increasing Cu doses, where 5.4 leaves per plant were counted at 400 mg Cu kg^{-1} compared to 4.5 leaves per control. In similar way, ESP mother plant had 6.2 leaves at 300 mg Cu kg^{-1} against 4.3 leaves per control. In contrast, BL mother plant significantly ($p < 0.05$) affected by increasing Cu doses, where control plant had 6.3 leaves while plant of treatment of 300 mg Cu kg^{-1} recorded 4.1 leaves but at highest Cu concentration BL plant had 5.7 leaves.

Table 6: Number of leaves of mother plant and new tillers of giant reed ecotypes at harvest

	Mother plant			New tillers		
Cu (mg kg^{-1})	*STM*	*BL*	*ESP*	*STM*	*BL*	*ESP*
0	4.5abc	6.3a	4.3abc	3.3c	4.1abc	4.1abc
100	4.2bc	4.8abc	3.9c	5.3a	4.4abc	5.0ab
200	4.8abc	5.9ab	4.2bc	4.4abc	4.4abc	4.4abc
300	5.3abc	4.1bc	6.2a	4.0bc	3.9bc	4.3abc
400	5.4abc	5.7abc	5.4abc	4.9ab	4.4abc	4.5abc

Different letters in same column show significant differences among each group of treatments according to Duncan's test at $p < 0.05$

Table 7: Length of internode of mother plant and new tillers of giant reed ecotypes at harvest

	Mother plant			New tillers		
Cu (mg kg^{-1})	*STM*	*BL*	*ESP*	*STM*	*BL*	*ESP*
0	16.7ab	10.0bc	13.7abc	21.4de	9.6g	30.9bc
100	17.7ab	12.8abc	17.1ab	30.0bc	13.9efg	40.8a
200	15.4abc	13.0abc	20.7a	25.2cd	12.6fg	36.8ab
300	13.5abc	7.9c	14.6abc	24.4cd	9.5g	15.4efg
400	12.1bc	8.6c	13.1abc	19.5def	9.0g	25.5cd

Different letters in same column show significant differences among each group of treatments according to Duncan's test at $p < 0.05$

There was consistency in data of number of leaves of new tillers with data of mother plants. Moreover, adding Cu to soil-like growth medium significantly ($p < 0.05$) induced the number of leaves of all three ecotypes compared to control plants. Highest recorded number of leaves of STM plant was measured at treatment of 100 mg Cu kg^{-1}, while BL and ESP plants had highest number of leaves at Cu concentrations of 400 and 100 mg kg^{-1}, respectively. However, number of leaves per plant is one of most important factors that have direct effects on biomass production of plant. Since giant reed is C_3 plant but it many literatures have reported that giant reed can behave as C_4 plant (Bell, 1997; Alshaal et al., 2014), therefore studying number and area of leaves is critical because it's important for photosynthesis and thus the amount of carbohydrates synthesized by plant mainly depends on it.

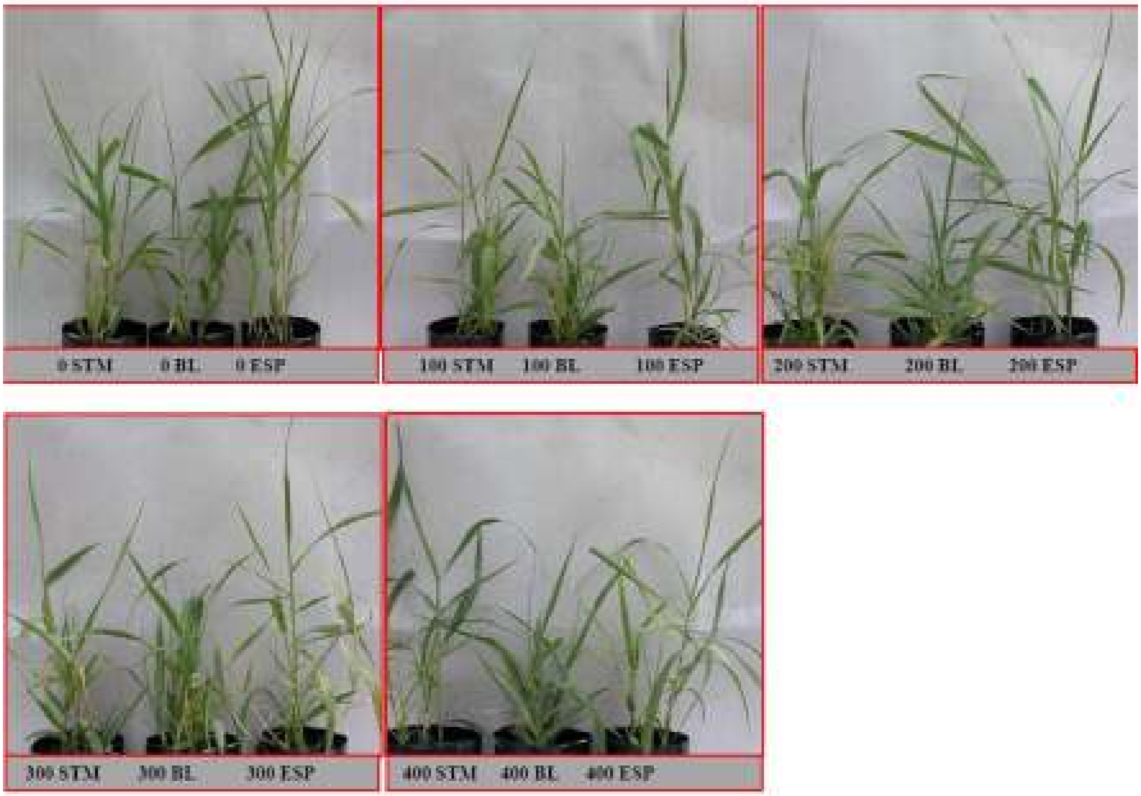


Fig. 13: Effect of elevated Cu concentrations on giant reed ecotypes (STM, BL, and ESP).

High Cu concentration i.e., 400 mg kg^{-1}, significantly ($p < 0.05$) reduced length of internodes of mother plants of STM and BL ecotypes compared to control plant. But ESP ecotype was less negatively affected with high Cu treatment, where the longest internode was noticed at treatment of 200 mg Cu kg^{-1} compared to control (Table 7). On the other hand, 100 mg Cu kg^{-1} significantly ($p < 0.05$) induced the length of internodes of new tillers among all ecotypes. While, longest internodes of three ecotypes were found at 100 mg Cu kg^{-1}, shortest internodes were recorded for all ecotypes at concentration of 400 mg Cu kg^{-1}.

4.2.2. Shoot and root length at harvest

At harvest of experiment, data of shoot length showed that plants of STM ecotype were more resistant to increasing Cu levels added to soil-like growth medium than BL and ESP ecotypes. Moreover, all Cu concentrations induced the shoot length of STM plants, where values of shoot length at all treatments of Cu were higher than that of control. However, shoot length of control plant was 31.38 cm while, at treatment of 400 mg Cu kg^{-1} 45.50 cm were recorded for shoot of STM plant. The highest measured value of STM shoot length was 47.38 cm at 100 mg Cu kg^{-1} (Fig. 14 and 15).

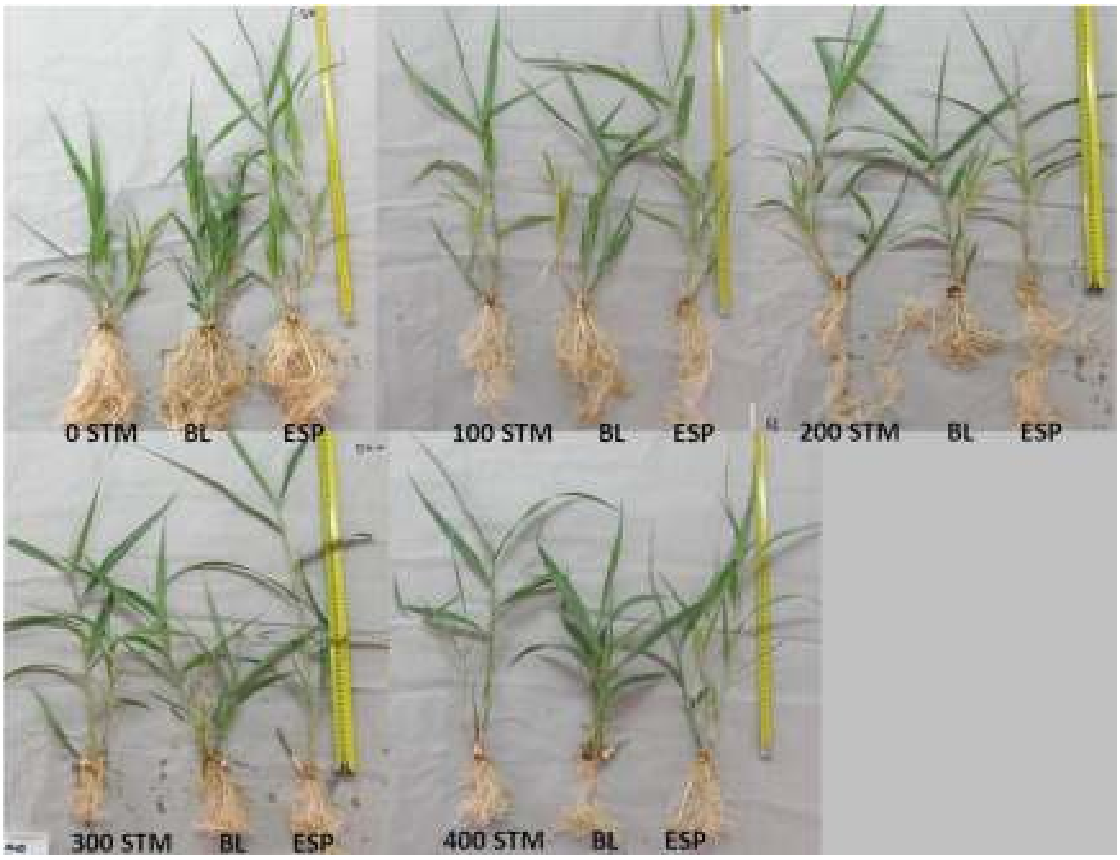


Fig. 14: Cu-toxicity symptoms on giant reed ecotypes (STM, BL, and ESP) grown on increasing Cu concentrations at harvest.

On the other hand, increasing Cu doses enhanced shoot length of BL ecotype up to 300 mg kg^{-1} compared to control plant, whilst concentration of 400 mg Cu kg^{-1} reduced shoot length recording the lowest value of shoot length among all treatments.

Shoot length of control plant was 30.83 cm, and significantly ($p < 0.05$) increased to maximum value of 36.25 cm at treatment of 200 mg Cu kg^{-1}. While shoot length at 400 mg Cu kg^{-1} was 29.88 cm (Fig. 14 and 15). These values might report that BL ecotype is tolerant for low concentrations of Cu up to 300 mg kg^{-1}, but higher Cu levels may lead to significant reduction in shoot length thus decrease the biomass production.

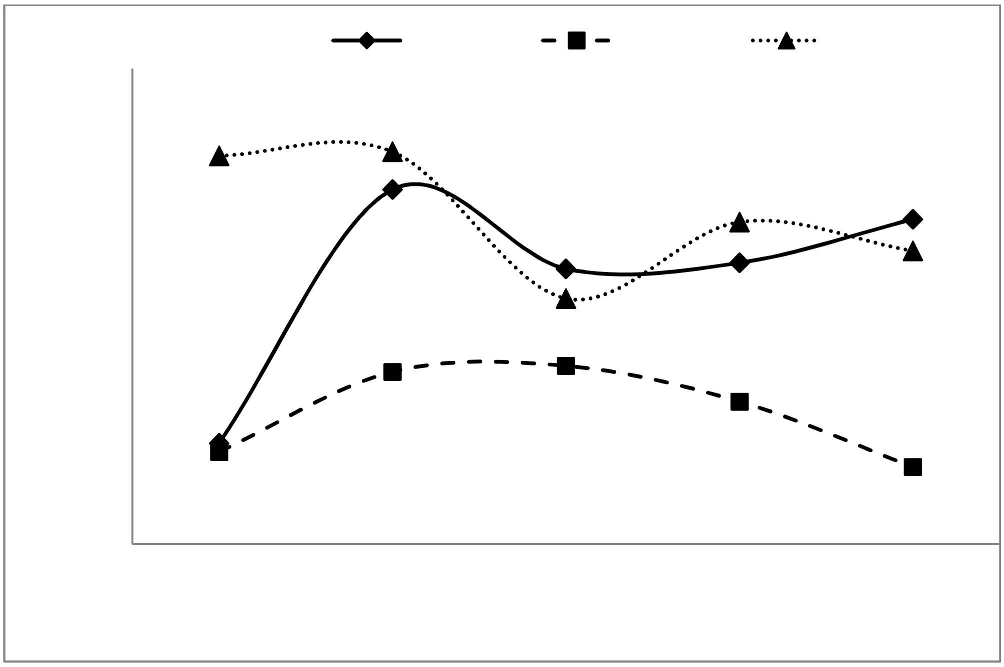


Fig. 15: Shoot length of giant reed ecotypes (STM, BL, and ESP) at harvest after growing on different concentrations of Cu. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

In contrast to STM and BL ecotypes, plants of ESP ecotype were sensitive to high Cu levels in soil-like growth medium. Increasing Cu doses over 100 mg kg^{-1} significantly reduced shoot length of ESP plants against control treatment. Highest Cu concentration, 400 mg kg^{-1}, decreased shoot length to 43.50 cm against 49.50 cm for control plant. However, shoot length was insignificantly reduced at treatment of 300 mg Cu kg^{-1} compared to no Cu treatment where shoot length was 45.33 cm (Fig. 14 and 15). However, no toxicity symptoms for elevated Cu concentrations were noticed on shoot parts of all ecotypes. Plants of giant reed ecotypes were healthy and no dwarfism, no bluish in color, no twisted leaves, no burned edges, and no chlorosis were found on shoots of STM, BL, and ESP ecotypes. The only noticeable effect of increasing Cu on plant growth was decrease of shoot length under some treatments (Fig. 14). Papazoglou et al. (2005) reported that irrigated growing giant reed plants with different levels of Cd and Ni for 2 years pot experiment did not significantly ($p < 0.05$) affect the stem height and other morphological characteristics.

On the other hand, toxicity symptoms of high Cu concentrations were clearly noticed on root system of all giant reed ecotypes. In general, with increasing Cu levels in soil-like growth medium root length was reduced significantly ($p < 0.05$) for all ecotypes, except STM root system at treatment of 100 mg Cu kg^{-1}. It recorded the highest value of root length, 35.13 cm, among all Cu treatments in all ecotypes while control plant of STM has length of 24.50 cm for root system. However, it could be concluded that length of root system of all three ecotype was severely reduced with treatments above 200 mg Cu kg^{-1}. Shortest root systems were measured at Cu concentration of 400 mg kg^{-1} in all ecotypes (Fig. 16).

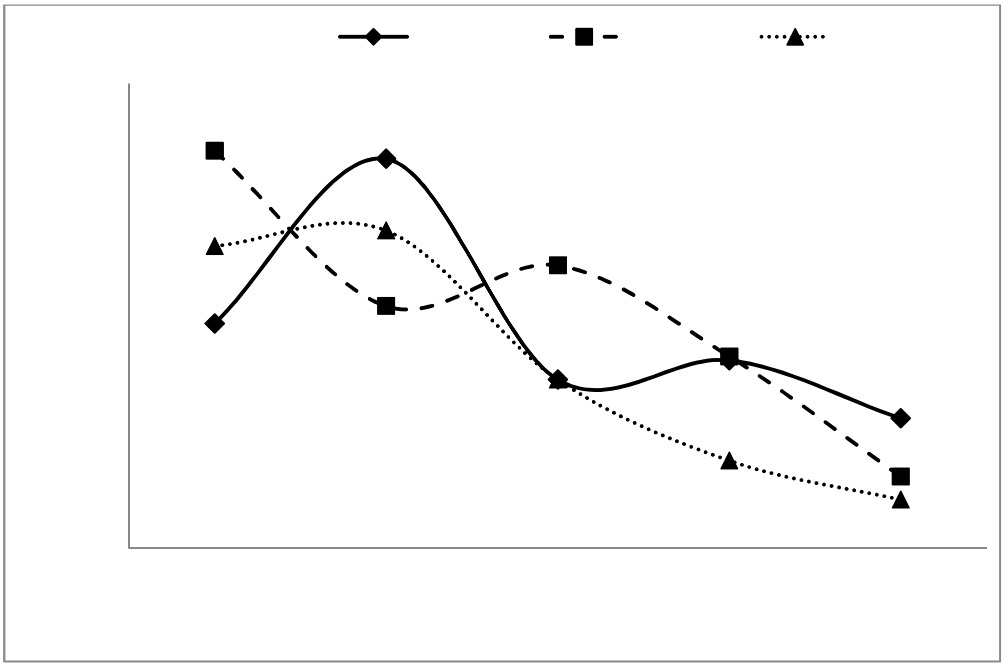


Fig. 16: Root length of giant reed ecotypes (STM, BL, and ESP) at harvest after growing on different concentrations of Cu. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

Adding Cu to the soil-like growth medium at increasing concentrations up to 100 mg kg^{-1} positively affected the growth and development of root systems of STM and ESP ecotypes but BL was negatively affected. Well developed and healthy root systems were seen; as well as root hairs was present in all ecotypes. In the lowest Cu concentrations, root systems were distinguished with fewer, longer roots, but in the high Cu concentrations above 200 mg kg^{-1} plants possessed more and shorter roots. In STM, BL, and 20SZ ecotypes the highest Cu concentration (400 mg kg^{-1}) inhibited the growth of root systems. Roots were slightly stunted, and the root cuticle was thickened, and brown, especially for the BL and ESP ecotypes. Neither lateral roots nor root hairs were clearly seen on the root systems of both ecotypes. STM plants seemed to be more tolerant to high Cu concentrations where, it recorded the higest values for root system among all investigated ecotypes (Fig. 17).

Fig. 17: Root system of giant reed ecotypes (STM, BL, and ESP) at harvest after growing on different concentrations of Cu.

4.2.3. Shoot and root volumes of giant reed ecotypes

Data of shoot volume of giant reed ecotypes at harvest of greenhouse experiment using different Cu concentrations is depicted in Fig. 18. Results of shoot volume of STM plants were in harmony with results of its shoot length. All Cu treatments induced the growth of STM plants and this reflected on its shoot volume.

Values of shoot volumes under Cu treatments were higher than that of control plants except treatment of 200 mg Cu kg^{-1}. Highest shoot volume, 6.25 ml, was found at treatments of 300 and 400 mg Cu kg^{-1} against 4.25 ml for shoot volume of control plant. Plants of BL ecotype had different response to Cu treatments, where low Cu concentrations, i.e., 100 and 200 mg kg^{-1} enhanced shoot volume values compared to control plants but with increasing Cu concnetrations above 200 mg kg^{-1} shoot volume was clearly reduced in compare to no Cu treatment. However, highest recorded value of shoot volume of BL ecotype (6.50 ml) was noticed at treatment of 100 mg Cu kg^{-1} while control plant had 5.25 ml for its shoot volume. On the other hand, ESP plants were more sensitive to adding Cu to soil-like growth medium, where shoot volume of all plants of Cu treatments were lower than that measured for control plant. While control plant had shoot volume of 8.50 ml, plant of 300 mg Cu kg^{-1} treatment had the highest value (7.33 ml) among all Cu treated plants.

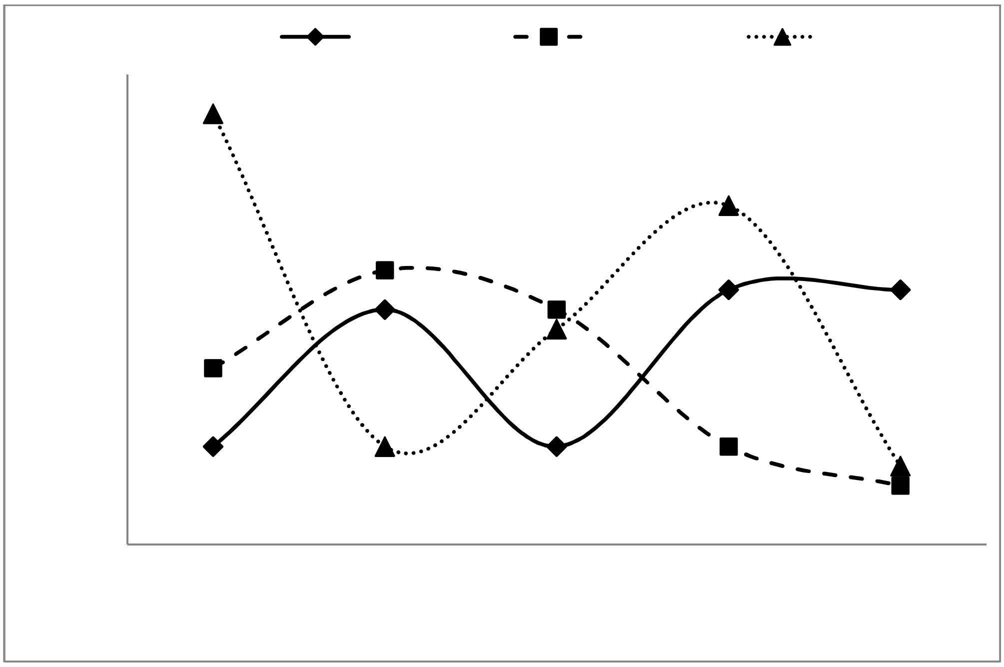


Fig. 18: Volume of **s**hoot part of giant reed ecotypes (STM, BL, and ESP) at harvest after growing on different concentrations of Cu. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

Figure 19 shows the measured data of volume of root systems of all three ecotypes of giant reed after growing on elevated Cu concentrations. Only BL plants had tendency for its root volume with increasing Cu levels in soil but both STM and ESP ecotypes recorded hesitated values for their root volumes. With increasing Cu doses volume of root system of BL ecotype significantly decreased. The highest measured number for root volume of BL plant was 8.50 ml for control plant, while lowest value was 4.75 ml at treatment of 300 mg Cu kg^{-1}. For STM and ESP ecotypes, although treatment of 200 mg Cu kg^{-1} increased the root volume of ESP plant than control plant achieving 7.00 ml against 6.50 ml for control, it reduced the volume of root system of STM plant recording the lowest value among all treatments (2.25 ml) when root volume of control plant had 4.50 ml. However, at the highest Cu treatment (400 mg kg^{-1}) STM and BL ecotypes showed more tolerance than ESP ecotype which recorded the worst value for root volume. Finally, it could be summarized that STM ecotype had longer root system but less dense with increasing Cu levels than BL plant that had opposite morphology for root system which was shorter but denser. ESP ecotype had the shortest and least dense root system when added Cu increased in soil-like growth medium.

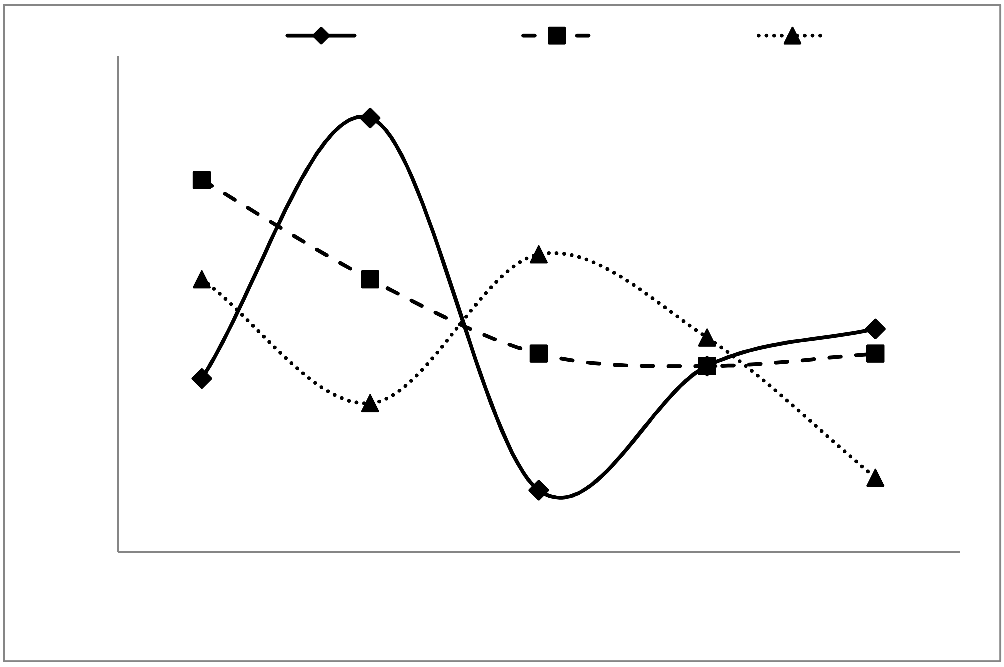


Fig. 19: Volume of root system of giant reed ecotypes (STM, BL, and ESP) at harvest after growing on different concentrations of Cu. Different letters on same line show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

4.2.4. Wet and dry masses of giant reed ecotypes

Recently, biomass crops have been proposed for phytoremediation purposes not because of its ability to absorb pollutants at high rates, but because of its high biomass production. Thus it would be faster to clean contaminated sites by establishment of biomass crops. Therefore, biomass production of giant reed growing on polluted soils is considered the key character that should be rely on in order to evaluate the possibility for using giant reed for phytoremediation purposes.

Root system of studied giant reed ecotypes was severely affected by increased Cu doses in soil-like growth medium, but such effects did not record on shoot system. Consequently, wet masses of root system of STM, BL, and ESP ecotypes in the same way decreased with increasing Cu levels. Wet masses of root systems of STM, BL, and ESP ecotypes were obviously reduced with increasing Cu in soil-like growth medium up to 300 mg kg^{-1}. But values of STM and BL ecotypes started to increase again at 400 mg Cu kg^{-1}, where values of wet masses of both ecotypes at highest Cu treatment were not the worst among all treatments (Fig. 20).

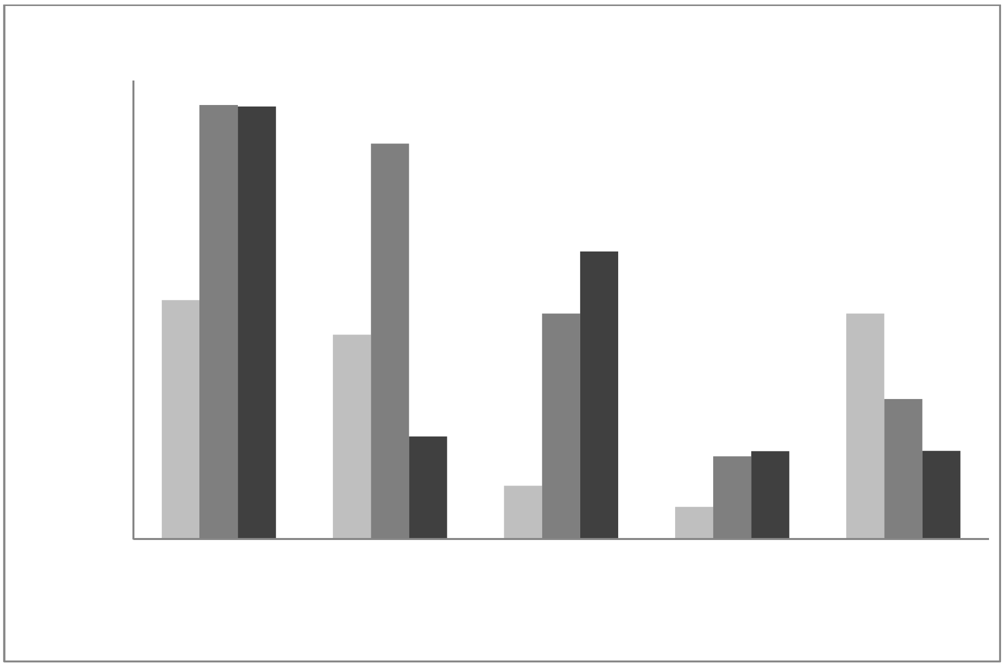


Fig. 20: Wet mass of root system of giant reed ecotypes (STM, BL, and ESP) at harvest after grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

However, STM plants had the lowest wet masses for its root system among all three ecotypes with increasing Cu concentrations up to 300 mg kg^{-1} but at 400 mg Cu kg^{-1} STM plants recorded the highest wet mass compared to BL and ESP ecotypes. Wet mass of BL root system was the highest at treatment of 100 mg Cu kg^{-1} while, at treatment of 200 mg Cu kg^{-1} ESP plants had the highest wet mass for its root system. From mentioned results above, it becomes clear that root system STM ecotype was the most tolerant ecotype for high Cu levels, where it had the longest root and biggest volume for root system beside the highest wet mass.

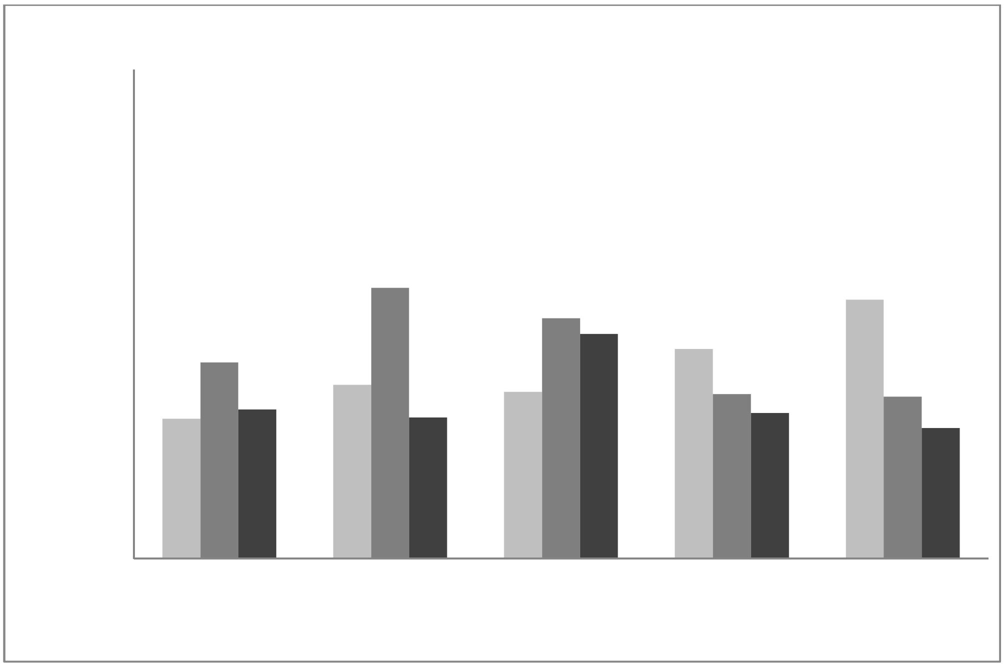


Fig. 21: Wet mass of culm of giant reed ecotypes (STM, BL, and ESP) at harvest after grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

Although, adding Cu to soil-like growth medium by increasing concentrations up to 400 mg kg^{-1}, which is above the normal levels of Cu in soils (Kabata-Pendias, 2011), no effects on wet masses of culm of all three ecotypes were noticed (Fig. 21). It observed that at low Cu concentrations (less than 200 mg Cu kg^{-1}) culm of BL ecotype had the highest values for wet mass among all investigated giant reed ecotypes. Wet mass of BL culm was 10.03, 13.84, and 12.28 g plant^{-1} at 0, 100, and 200 mg Cu kg^{-1}, respectively, but with increasing Cu levels wet mass recorded 8.41 and 8.28 g plant^{-1} when Cu concentration was 300 and 400 mg kg^{-1}. On the other hand, culm of ESP ecotype had no obvious trend with increasing Cu doses, where it kept almost same values of wet mass of culm at all Cu treatments except treatment of 200 mg kg^{-1} which had the highest measured value of wet mass (11.49 g $plant^{-1}$). Interestingly, STM plant culm had nearly same values of wet mass at low Cu concentrations up to 200 mg kg^{-1} ranging from 7.15 to 8.52 g $plant^{-1}$ at 0 and 200 mg kg^{-1} treatments. But increasing Cu above 200 mg kg^{-1} enhanced the wet mass of culm, where 10.71 and 13.24 g $plant^{-1}$ were measured for wet mass of culm at treatments of 300 and 400 mg kg^{-1}, respectively (Fig. 21). However, STM plant showed positive response towards high Cu concentrations, this might explain that STM ecotype is more suited than BL and ESP ecotypes for phytoremediation of Cu-contaminated site above 200 mg kg^{-1}.

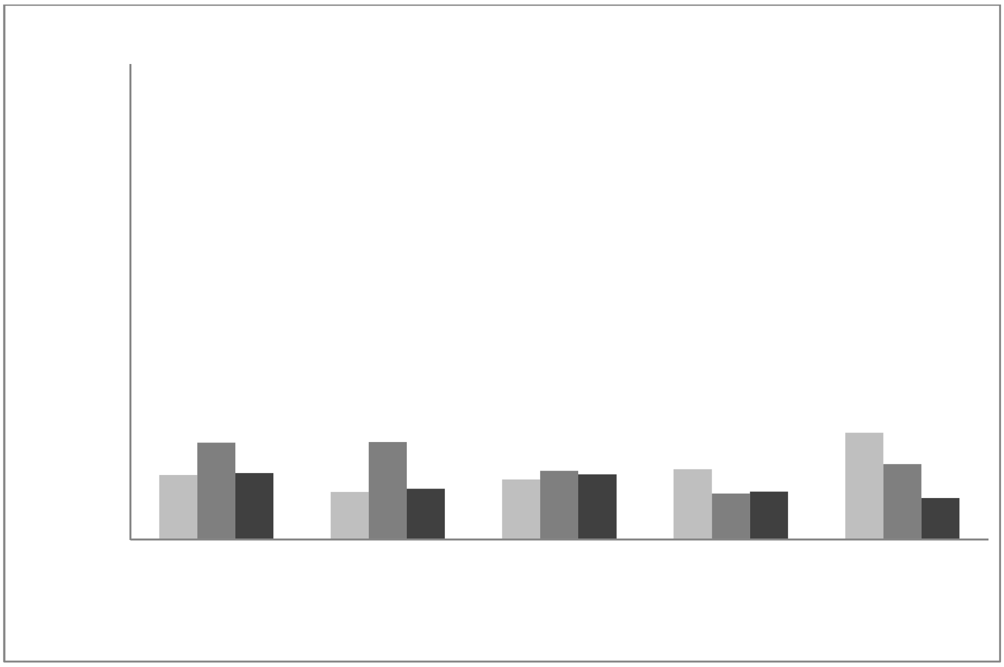


Fig. 22: Wet mass of leaf blade of giant reed ecotypes (STM, BL, and ESP) at harvest after grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

Wet masses of leaf blade of giant reed ecotypes are pictured in Fig. 22. In the same way with wet masses of plant culm, results of leaf blade wet mass of ESP, BL, and ESP ecotypes were. BL plants had the highest values for wet mass of leaf blade at treatments of 0, 100, and 200 mg Cu kg^{-1}, but when Cu doese increased in soil-like growth medium STM leaf blade measured the highest values among othe ecotypes. This emphasizes that STM ecotype is resistant to high Cu doses above 200 mg kg^{-1}.

However, ESP ecotype kept the same tendency for wet mass of its leaf balde as in its wet mass of culm, where it had same values among all treatments. In general, the highest value of wet mass of leaf blade among all treatments and ecotypes was recorded for STM ecotype at the highest treatment of Cu (400 mg kg^{-1}).

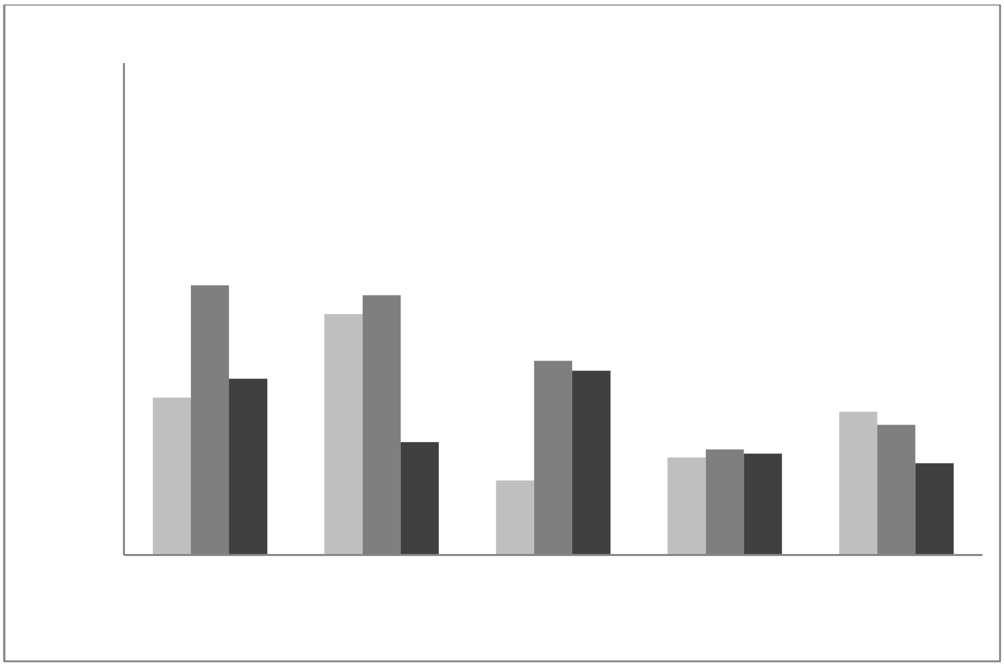


Fig. 23: Dry mass of root system of giant reed ecotypes (STM, BL, and ESP) at harvest after grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

In general, all three ecotypes had no clear tendency for dry mass of their root system towards increasing Cu levels in soil-like growth medium used for experiment (Fig. 23). However, BL plant showed negative response towards increasing Cu doses, where with increase added Cu the dry mass of root system gradually reduced till treatment of 300 mg kg^{-1} but increased again at 400 mg Cu kg^{-1}. Dry mass of BL ecotype decreased from 3.29 to 1.29 g $plant^{-1}$ when Cu concentration changed from 0 to 300 mg kg^{-1}. Moreover, in all treatments up to treatment of 400 mg kg^{-1} BL root system had the highest values among all treatments. Unlike BL ecotype, STM and ESP ecotypes had hesitated values for dry mass of root system with elevated Cu doses. For STM ecotype, treatment of 100 mg kg^{-1} of Cu enhanced dry mass of root system, while other treatments had lower values of dry mass compared to control plant. ESP plants recorded the highest dry mass values at treatment of 200 mg kg^{-1}, and lowest measured dry mass of its root system was found at treatment of 400 mg kg^{-1}. However, at the highest Cu dose in soil-like growth medium STM plant possessed the highest dry mass of root system among all ecotypes (Fig. 23).

In contrast to dry mass of root system of studied giant reed ecotypes, dry mass of culm was positively affected with increasing Cu doses (Fig. 24). All ecotypes had higher values of dry mass of culm compared to control plants. With increasing Cu levels in soil-like growth medium dry mass of culm of STM, BL, and ESP increased signficantely. The highest dry mass of BL culm was 5.45 g $plant^{-1}$ and was achieved at treatment of 100 mg kg^{-1}, while highest value of STM culm was 4.81 g $plant^{-1}$ and is found when Cu concentration in soil-like growth medium was 300 mg kg^{-1}. But ESP plants had highest dry mass of culm at treatment of 200 mg Cu kg^{-1} and it recorded 5.20 g $plant^{-1}$ (Fig. 24). Interestingly, under low Cu concentrations in soil-like growth medium both BL and ESP ecotypes had higher values of dry mass of plant culm compared to STM plants. At Cu concentrations more than 200 mg kg^{-1}, while STM plants possessed increased and highest values of dry mass of culm, dry mass of culm of BL and ESP sharply decreased with increasing Cu levels. These findings emphasize that STM ecotype is more tolerant for higher Cu levels than 200 mg kg^{-1}, therefore STM might be recommended to phytoremediation purposes in extremely contaminated sites with Cu.

Dry masses of leaf blade of STM, BL, and ESP ecotypes are presented in Fig. 25. Whilst, STM plants had lowest dry mass of leaf blade among all giant reed ecotypes, in higher Cu treatments above 200 mg kg^{-1} it had the highest dry mass compared to BL and ESP ecotypes. However, all ecotypes showed significant effects towards increasing Cu, where they measured higher values of leaf blade dry mass compared to control. In lower Cu concentrations than 300 mg kg^{-1}, BL and ESP plants had higher dry masses than STM plant, but BL ecotype was the highest ecotype in dry mass of leaf blade in Cu treatments up to 200 mg kg^{-1}. Highest dry masses of STM, BL, and ESP were 2.96, 3.01, and 2.60 g $plant^{-1}$ at treatments of 300, 100, and 200 mg kg^{-1}, respectively. These findings were different than those of Sheldon and Menzies (2005) who reported low Cu concentrations between 0.2 and 1 μM significantly reduced root growth and disrupted the root cuticle of Rhodes grass.

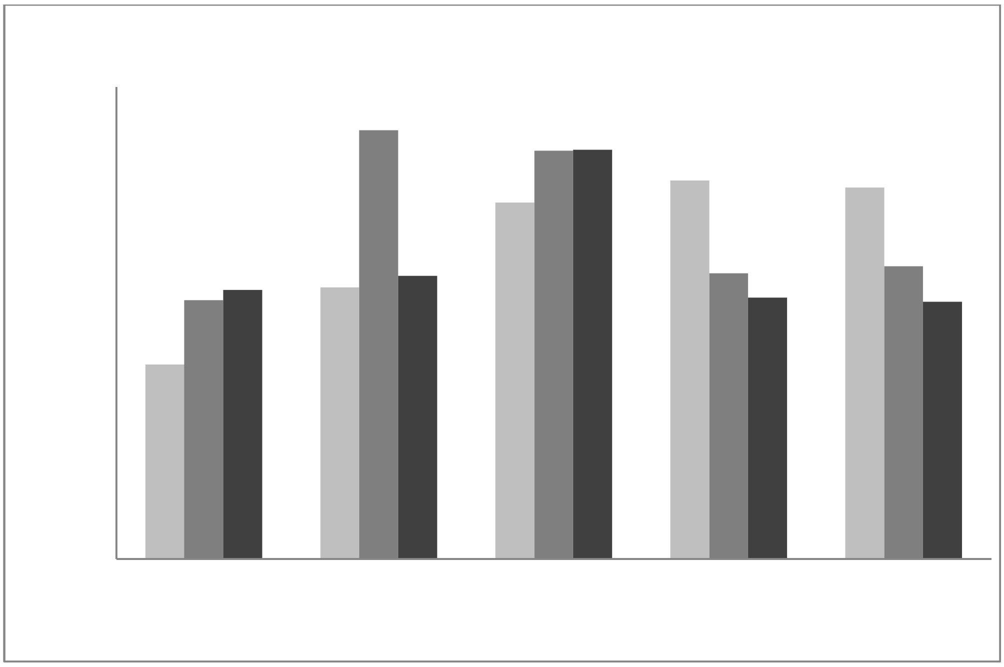


Fig. 24: Dry mass of culm of giant reed ecotypes (STM, BL, and ESP) at harvest after grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

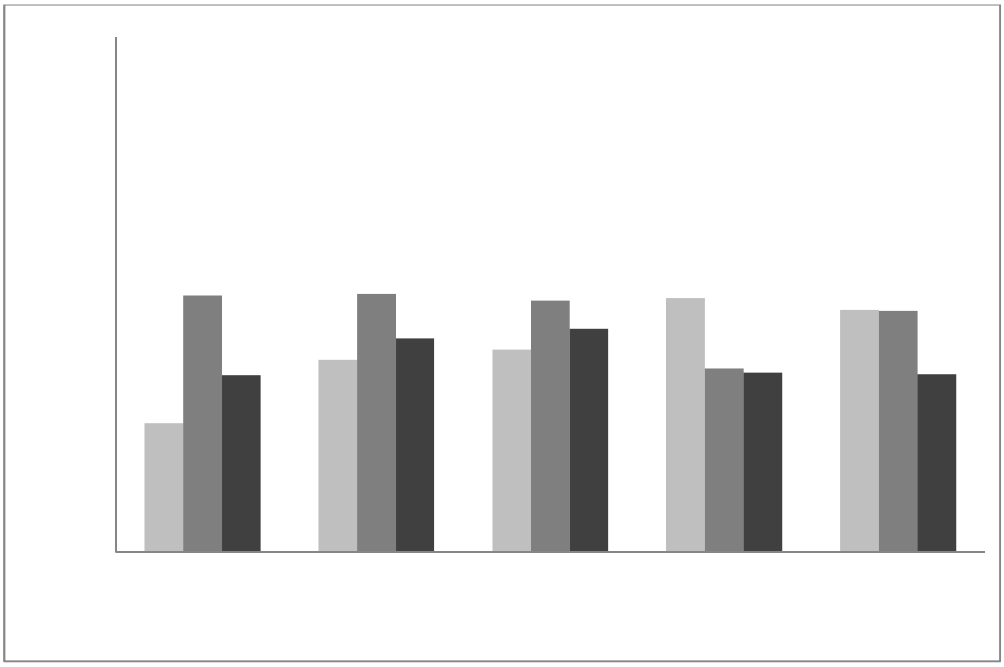


Fig. 25: Dry mass of leaf blade of giant reed ecotypes (STM, BL, and ESP) at harvest after grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

From all mentioned vegetative results above, it could be concluded that the morphological characteristics as well as wet and dry masses of studied giant reed ecotypes (STM, BL, and ESP) did not show any detrimental or toxic effects of Cu exposures. This might be attributed to *hormesis* “using a low dose of any toxin - that causes harmful effects for organisms when apply in high doses – stimulates adaptive beneficial effects on the cell or organism (Calabrese, 2004; Mattson, 2008)”. Since Cu is an essential micronutrient element, therefore, at low doses it induces the plant growth. In the current study, the aboveground part of giant reed ecotypes did not significantly affect with high Cu doses. It showed positive effects at highest Cu treatments (400 mg kg^{-1}), so as this could be stimulated dose of Cu for shoot part growth. In contrast, root system negatively affected with increasing Cu exposures, so 200 mg Cu kg^{-1} could be considered as threshold dose of Cu for root growth.

4.2.5. Cu content in different plant parts of giant reed ecotypes

Giant reed grass once established, it can re-grow for many years without new plantations because it produces many new tillers during its live. The new tillers are characterized by their stronger growth than their mother plant, as well as they have thicker and longer culms compared to primary plant. Therefore, one of the proposed tolerance mechanisms for heavy metals is that mother plant, which is directly transplanted on metal-contaminated sites and firstly faces the pollutants at the highest rates, can accumulate and trap the pollutants throughout its tissues then avoid them from the new tillers.

Consequently, total Cu content in shoot part of mother plant was measured (because separation of root system of mother plant from new tillers was impossible) and is depicted in Fig. 26. Significant differences ($p < 0.05$) of Cu contents of mother plant of all three studied ecotypes (STM, BL, and ESP) were found with increasing Cu in soil-like growth medium. Mother plant of STM ecotype had the highest content of Cu in its shoot parts compared to other two ecotypes under treatments of 100 and 300 mg Cu kg^{-1}, respectively. However, the highest Cu content in shoot part of mother plant of all ecotypes was 121 µg g^{-1} DM and found in STM plant at treatment 300 mg kg^{-1}. While Cu content in STM mother plant declined to 66 µg g^{-1} DM with increasing Cu in soil-like growth medium up to 400 mg kg^{-1}, ESP mother plant possessed the highest content of accumulated Cu in its shoot tissues compared to STM and BL ecotypes. In general, all ecotypes had increasing content of Cu in their tissues with elevating Cu level in soil-like growth medium. Whereas, highest Cu content of Cu in mother plant of STM and BL ecotypes was found at treatment of 300 mg kg^{-1}, ESP plant had the highest Cu content at treatment of 400 mg kg^{-1}.

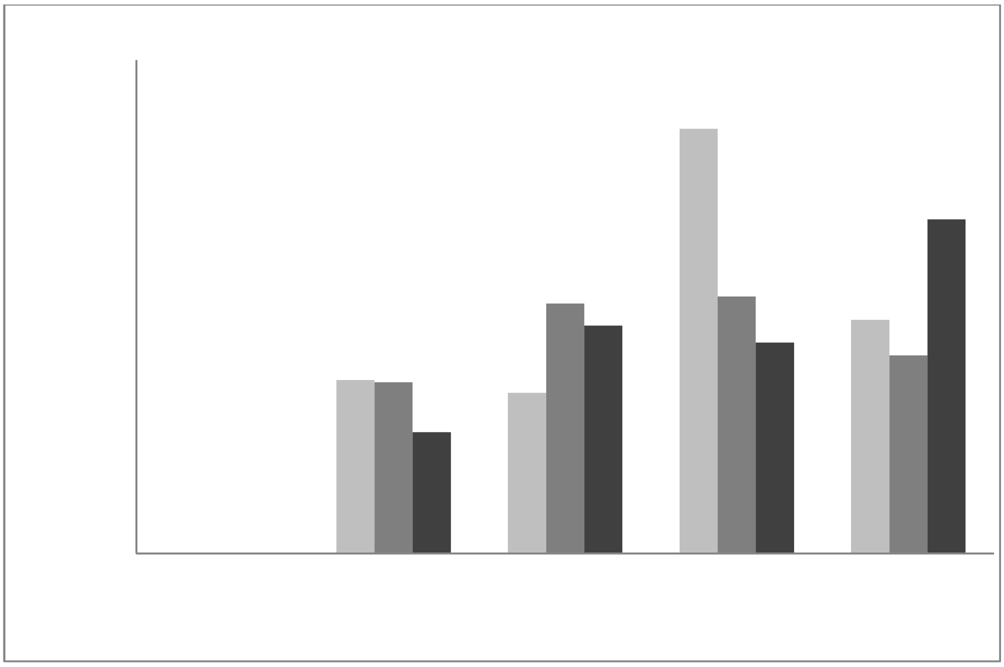


Fig. 26: Cu content of mother plant shoots of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

Cu content in root system of investigated STM, BL, and ESP ecotypes of giant reed significantly increased with increasing Cu concentrations in experimental pots as shown in Fig. 27. However, all ecotypes accumulated increasing amounts of Cu within their root systems with increasing Cu level in treatments, where Cu content in root system at treatment of 400 mg Cu kg^{-1} was 3 folds more than Cu content in root system of studied ecotypes at treatment of 100 mg Cu kg^{-1}. Plants of ESP ecotype showed higher ability to uptake Cu from contaminated soil, where it had the highest content of Cu in root system compared to other ecotypes at treatments of 100, 300, and 400 mg kg^{-1}, achieving 549, 1631, and 1829 µg g^{-1} DM. At highest Cu treatment (400 mg kg^{-1}) STM plant accumulated 1191 µg g^{-1} DM higher than BL ecotype that trapped 935 µg g^{-1} DM but both were lower than that accumulated within root system of ESP plant. The high Cu content in root system of ESP ecotype could be due its low dry mass of root system which means that high values for Cu content within low dry mass. On the other hand, root system of STM ecotype accumulated high percentages of Cu ranged from 71 to 95 % at treatments of 100 and 400 mg kg^{-1}, respectively, with average of 85 %. While, BL root system captured Cu within its tissues by 78 to 89 % from total Cu which uptake by plant, ESP plant accumulated in its root system almost between 88 to 96 % with average of 91 %. This means in order to ensure complete cleaning for Cu contaminated soil, giant reed ecotypes have to be taken off with its root system from soil after phytoremediation (however, it is difficult and uneconomic to remove the total root system of giant reed planst, since it has extended network system of rhizomes) because the highest accumulated amount of Cu will be in root system. This also could explain why no foliar symptoms of Cu toxicity had been noticed on shoot system of giant reed ecotypes which used in this current study.

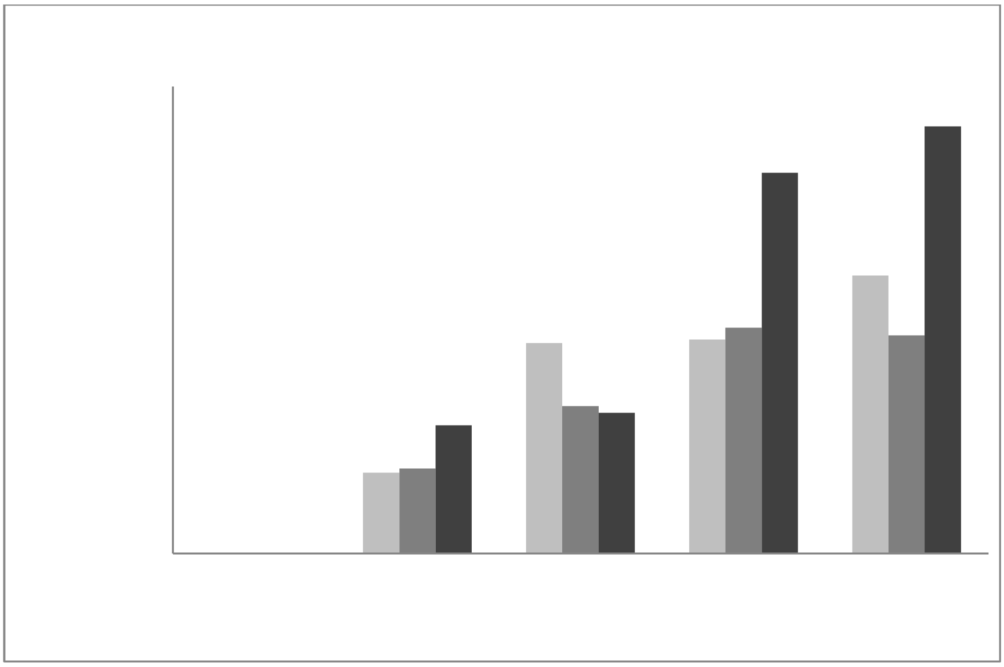


Fig. 27: Cu content of root system of giant reed ecotypes (STM, BL, and ESP) grown on different Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

Cu content of culm of ESP plant was directly proportional to Cu doses in soil-like growth medium as seen in Fig. 28. As Cu increased in the treatments, culm of ESP plant concentrated more Cu in its tissues ranging from 43 to 101 µg g^{-1} DM at treatments of 100 to 400 mg Cu kg^{-1}, respectively. However, the highest content of Cu (130 µg g^{-1} DM) among all ecotypes was recorded within culm of BL ecotype at treatment of 200 mg kg^{-1}. STM plant had highest Cu content (113 µg g^{-1} DM) in its culm at treatment of 100 mg kg^{-1}. Generally, both STM and BL ecotypes did not show obvious trend towards increasing Cu in treatments, where hesitated tendency was noticed. Even so, STM culm had low Cu content compared to other tested ecotypes; this could be a consequence for its high dry mass with regared to dry mass of culm of BL and ESP ecotypes. Despite, giant reed plants showed considerable capacity to uptake and remove toxic metals like Cu from metal-contaminated environemnts (Alshaal et al., 2013; Papazoglou et al., 2005; Nsanganwimana et al., 2014), but most of toxic metals accumulate in root system. Simon et al. (2011) documented aboveground part of giant reed plants collected low Cu content, when it grew on soil amended with municipal sewage sludge compost (MSSC) which is moderately contaminated with Cu (140 mg kg^{-1}). Therefore it would be safe to burn aboveground part of giant reed plants for heating purposes after growing on metal-contaminated sites (Simon et al., 2011).

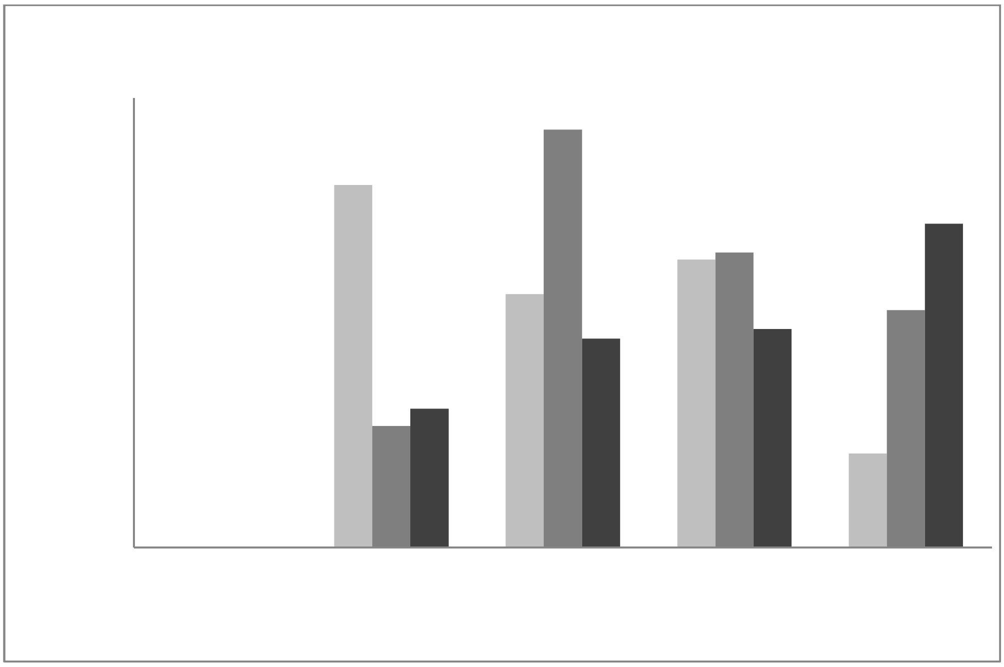


Fig. 28: Cu content of culm of giant reed ecotypes (STM, BL, and ESP) grown on different Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

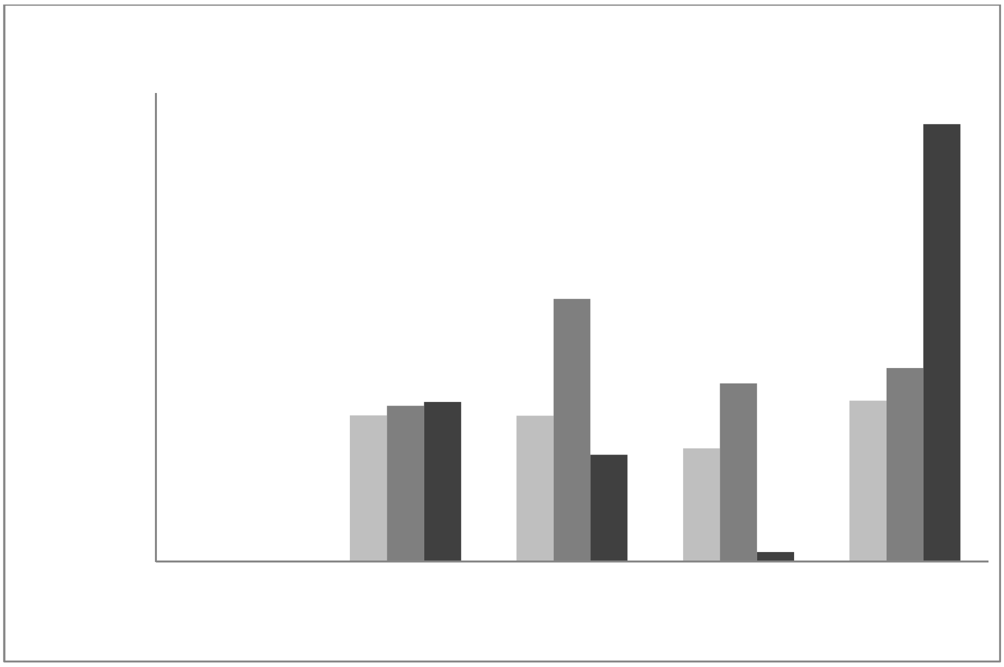


Fig. 29: Cu content of leaf blade of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

Basically, increasing Cu levels did not substantially increase the contents of Cu in leaf blade of all investigated ecotypes, except ESP plant which had the highest recorded Cu content (84 µg g^{-1} DM) at highest Cu treatment, i.e., 400 mg kg^{-1}. However, at treatment of 300 mg Cu kg^{-1} leaf blade of ESP ecotype had the lowest measured value of Cu content (2 µg g^{-1} DM) among all ecotypes, but this could be due do mistake in sample preparation for measurements (Fig. 29). Broadly, significant differences were noticed between ecotypes within treatments, but no clear trend for increasing Cu on content of Cu in leaf blade. At treatments of 200 and 300 mg kg^{-1} BL ecotype recorded the highest Cu content in tissues of its leaf blade; likewise ESP ecotype had the highest Cu content at treatments of 100 and 400 mg kg^{-1}. Unusually, Cu content in leaf blade of STM decreased with increasing Cu concentrations in soil-like growth medium up to 300 mg kg^{-1}, but at treatment of 400 mg kg^{-1} it increased again achieving the highest Cu content in leaf blade among all treatments for STM ecotype (Fig. 29). This might refer to high dry mass of STM leaf blade that makes dilution for the Cu content in plant tissues. Simon et al. (2013) reported that using soil amendments such as ammonium nitrate (AN), municipal biocompost (MBC) and municipal sewage sludge compost (MSSC) did not significantly ($p < 0.05$) affect the uptake of Cu by giant reed plants and its accumulation in leaves against control. Leaves of giant reed plants accumulated much Cu than shoot parts of same plants (Simon et al., 2013); this was in contrast with that found in the current study.

4.2.6. Available and total Cu concentrations in soil-like growth medium

Effectively, adding Cu to soil-like growth medium as increasing doses resulted in increasing the concentration of available Cu in soil-like growth medium after harvesting of giant reed ecotypes. Basically, increasing available Cu concentrations were measured in pots after experiment under STM, BL, and ESP ecotypes. Significantly, available Cu concentration was increased gradually in pots after STM plant from 12 to 69 mg kg^{-1}, while in case of ESP plant available Cu level changed from 13 to 78 mg kg^{-1} at treatments of 100 to 400 mg kg^{-1}, respectively, for both ecotypes as shown in Fig. 30. On the other hand, BL ecotype caused gradual increase for available Cu concentration up to treatment of 300 mg kg^{-1}, but no increase in available Cu was measured at treatment of 400 mg kg^{-1} in BL pots. Anyway, with increasing Cu in experimental soil from 100 to 300 mg kg^{-1} STM ecotype's pot recorded the highest available Cu concentration among all ecotypes, but at 400 mg Cu kg^{-1} the highest available Cu concentration was found in pots of ESP ecotype.

Although, available Cu concentration increased in experimental pots of all ecotype with increasing treatments up to 400 mg kg^{-1} recording highest value of 78 mg kg^{-1}, but this value is still within the acceptable rangs of Cu in soil. Kabata-Pendias (2011) reported that natural Cu concentration in soils rangs between 2 – 109 mg kg^{-1}. Therefore, giant reed ecotypes mainly declined the concentration of Cu in all treatments compared to initial added Cu to experimental pots. Initially, Cu was added to experimental pots as $CuSO_4{\cdot}5H_2O$ which is soluble (available) form. And if we take in our consideration that no leaching from pots was allowed, since fully closed bottom pots were used in current experiment. Additionally, irrigation of experimental pots was done using distilled water and kept at water holding capacity to prevent any leaching with purpose to keep the initial added Cu concentration as it is without any losing. From all of the above, it could be concluded that, all investigated ecotypes succeeded to treat and decontaminate Cu-polluted sites efficiently, where after almost 3 months Cu levels in soil-like growth medium dropped down the toxic levels.

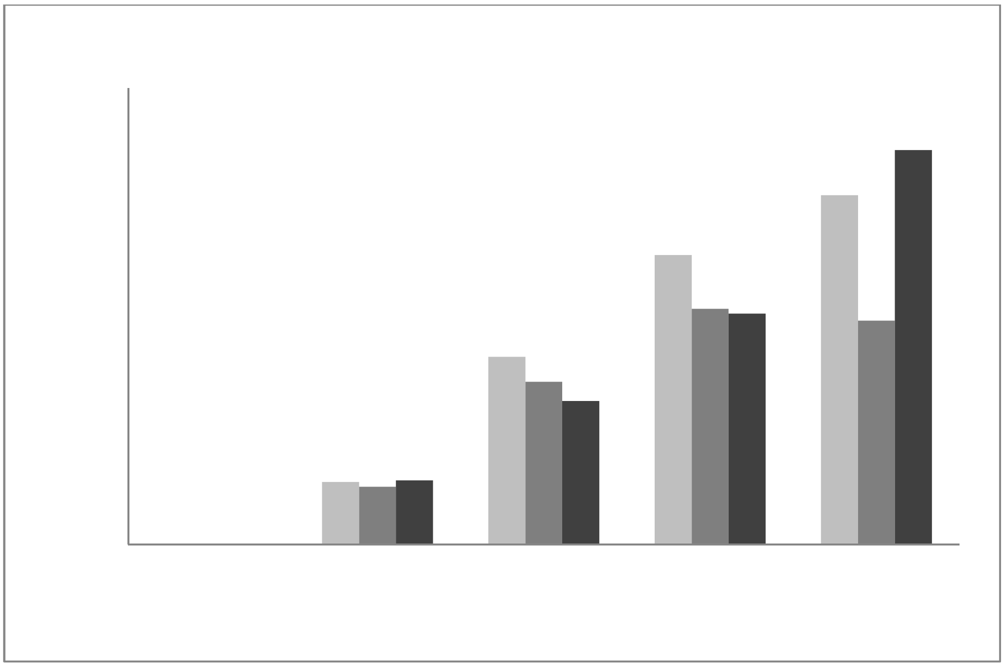


Fig. 30: Available Cu concentration in experimental pots of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

Data of total Cu concentrations in experimental pots after harvest of giant reed ecotypes growing on elevated Cu concentrations is portrayed in Fig. 31. Substantially, increasing Cu concentrations in soil-like growth medium resulted in significant increase of total concentrations of Cu in all pots under all ecotypes. Effect of giant reed ecotypes on total Cu concentration was clearly noticed. However, no significant differences for total Cu concentration were measured at treatments of 100 and 200 mg Cu kg^{-1} for ESP plants, but with increasing Cu in treatments exponential increase was found. Total Cu concentrations were 48, 154, and 175 mg kg^{-1} at treatments of 200, 300, and 400 mg Cu kg^{-1}, respectively. Similary, BL ecotype had same effect towards elevated Cu concentrations compared to ESP ecotype. In low treatments of Cu, total Cu concentration significantly changed from 34 to 56 mg kg^{-1} at treatments of 100 and 200 mg Cu kg^{-1} for BL plants. But at treatments of 300 and 400 mg Cu kg^{-1}, total Cu concentrations in experimental pots of BL ecotype were 136 and 149 mg kg^{-1}. STM ecotype was in contrast to BL and ESP ecotype towards elevated Cu concentrations in soil-like growth medium. However, total Cu concentration in pots of STM ecotype increased gradually with increasing Cu treatments, but this increase was almost less than half of that measured with BL and ESP ecotype. Total Cu concentrations were 32, 43, 77, and 75 mg kg^{-1} at treatments of 100, 200, 300, and 400 mg Cu kg^{-1} for STM plant. Clearly, it could be concluded that investigated giant reed ecotypes had different response towards increasing Cu concentrations in soil-like growth medium where they grew on. While, BL and ESP ecotypes tended to avoid toxicity of elevated Cu levels by eliminating Cu from soil-like growth medium by converting soluble Cu forms into insoluble forms (phytostablization), STM had different mechanism. However, STM tolerated high Cu concentrations by uptake it into its tissues where, it had the lowest values for insoluble Cu forms among studied giant reed ecotypes.

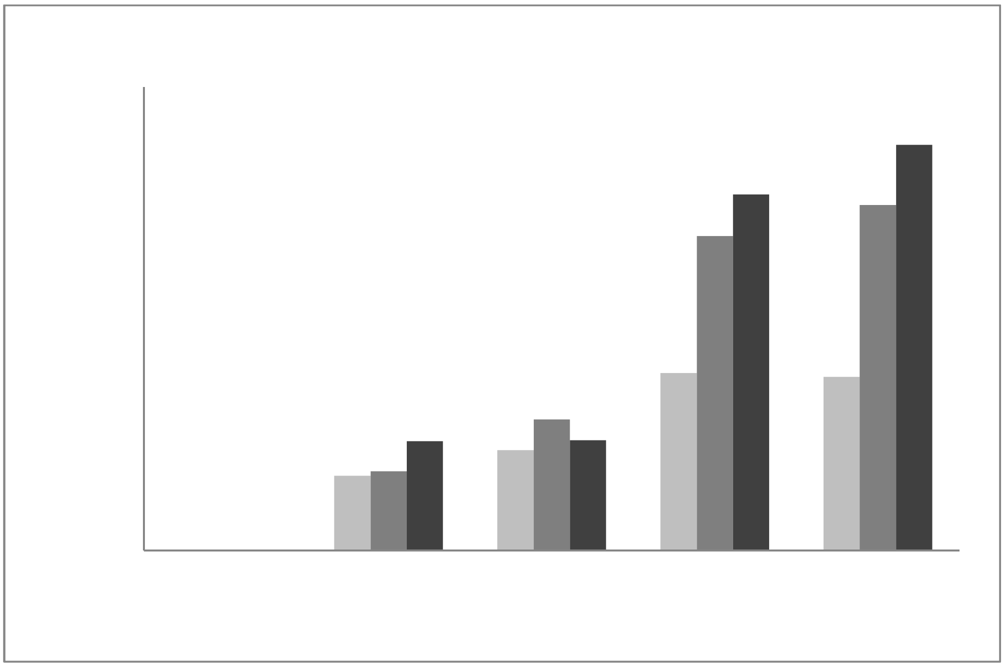


Fig. 31: Total Cu concentration in experimental pots of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

As consequence for preventing leaching from experimental pots during experiment period and keeping irrigation at water holding capacity, it could be explained that giant reed ecotypes had 2 possible mechanisms towards added Cu into their soil-like growth medium. Firstly, uptake Cu and bioconcentrate it throughout their living tissuses, secondly convert available added Cu into insoluble forms (phytostablization). Therefore, the fate of added Cu into experimental pots could classified into 3 types as follow; 1) uptake by plant, 2) converted into insoluble Cu forms (phytostablization), and 3) remained as available Cu form in soil-like growth medium. So that, we can use the following equation in order to investigate and interpret the effect of giant reed ecotypes on added Cu into soil-like growth medium, as well as to evaluate their efficiencies towards Cu phytoremediation.

Total Cu in soil = uptake Cu + phytostabilized Cu + remaining available Cu

Regarding the uptake of Cu by giant reed plants, STM ecoptye had the highest values of uptake Cu compared to BL and ESP ecotypes at all treatments. With increasing Cu concentrations in soil-like growth medium, Cu content in STM plant increased gradually. Contents of 68, 157, 223, and 325 µg g^{-1} were found in STM plant at treatments of 100, 200, 300, and 400 mg Cu kg^{-1}. On the other hand, BL ecotype recorded higher values of Cu content in plant tissues compared ESP ecotype (Fig. 32). BL plant accumulated 66, 144, 164, and 251 µg g^{-1} of Cu, while ESP ecotype bioconcentrated Cu by some of 53, 152, 146, and 225 µg g^{-1} at treatments of 100, 200, 300, and 400 mg Cu kg^{-1}, respectively. From above mentioned results, it could summarized that STM ecotype is more tolerant to high Cu levels and it recommended as phytoremediation candidate efficiently compared to BL and ESP ecotype which tended to phytostabilize Cu in soil-like growth medium than uptake it.

Among investigated ecotypes of giant reed, STM ecotype had the lowest values of stabilized Cu in soil at all treatments. Significant differences were measured for STM compared to BL and ESP especially at high Cu treatments, i.e., 300 and 400 mg kg^{-1}. While STM plant stabilized 6 mg kg^{-1} of Cu, BL and ESP plants stabilized 105 and 97 mg kg^{-1}, respectively, at treatment of 400 mg Cu kg^{-1}. However, no big differences were measured between BL and ESP ecotypes at all treatments, but with increasing Cu in soil-like growth medium above 200 mg kg^{-1}, the phytostabilized amount of Cu by BL and ESP plants exponentially increased. At treatment of 200 mg Cu kg^{-1}, BL and ESP plants stabilized 24 and 19 mg kg^{-1}, respectively, whereas, at 400 mg Cu kg^{-1} they stabilized 105 and 97 mg kg^{-1}, respectively, (Fig. 33).

Total removed Cu amount (mg) on dry biomass basis was calculated. 9.7 mg Cu was found in harvested dry biomass of STM plants at 100 mg Cu kg^{-1}, while this amount increased to 10, 10.2, and 13.9 mg Cu at 200, 300, and 400 mg Cu kg^{-1}, respectively. The majority of this amount was concentrated in root system of STM plants, where 69.2 to 90.2 % were recorded in root system.

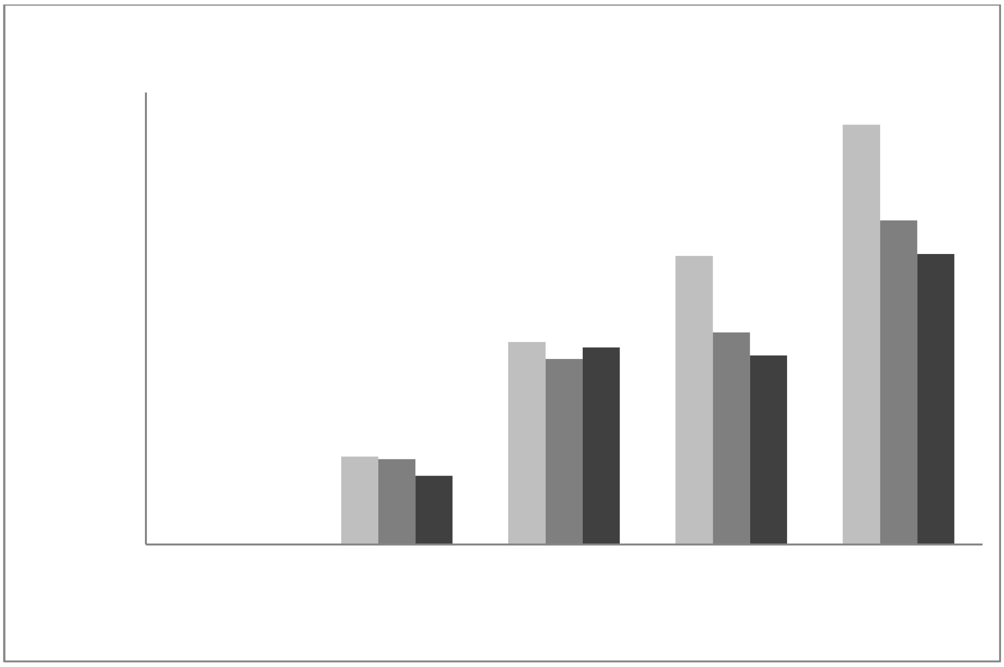


Fig. 32: Uptake Cu by giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

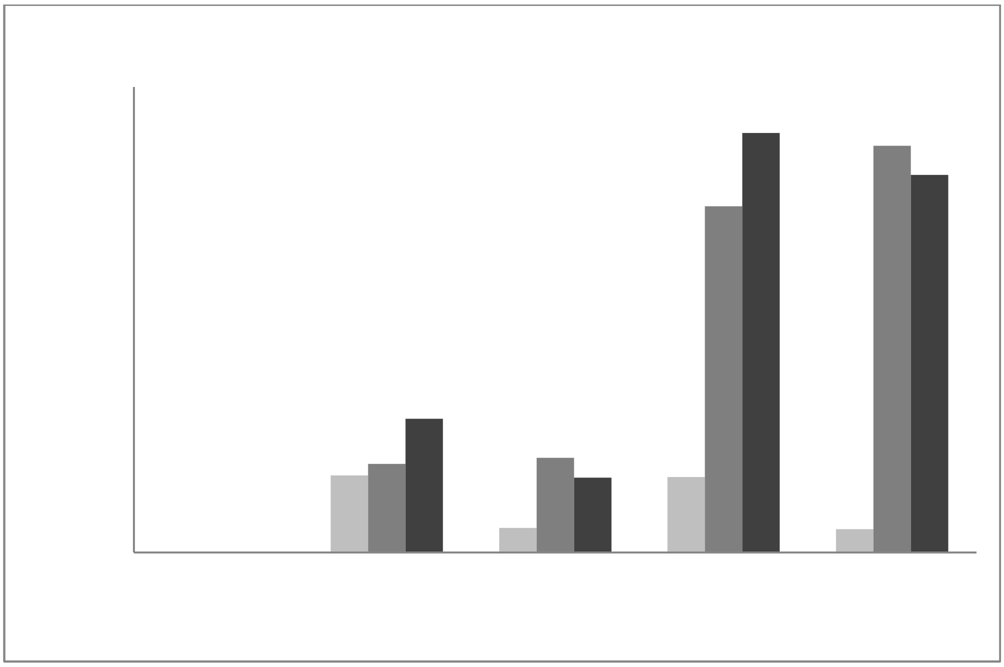


Fig. 33: Phytostabilized Cu by giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

BL plants accumulated much Cu within its dry biomass, where 11.3, 14.4, 12.4, and 12.3 mg Cu were measured at treatments of 100, 200, 300, and 400 mg Cu kg^{-1}. Root system of BL plants contained less Cu compared to STM root system, where 64.5 to 79.6 % of total removed Cu were found in root system of BL plants. At different Cu treatments, between 76.5 to 89.8 % of uptake Cu were calculated in harvested dry biomass of ESP plants. ESP plantes acquired 5.5, 9.8 12.6, and 13.3 mg Cu at 100, 200, 300, and 400 mg Cu kg^{-1}, respectively.

4.2.7. Cu uptake and phytoremediation indices

Transportation (TF) and bioconcentration (BCF) factors as well as removal rate (RR) were determined for STM, BL, and ESP ecotypes growing on increasing Cu concentrations with aim to evaluate the efficiency of Cu uptake and tolerance capacity of studied giant reed ecotypes. Then, determining whether these ecotypes could be exploiting for phytoremediation purposes or not.

Basically, calculated values of TF significantly affected with increasing Cu concentrations in soil-like growth medium. All TF values for STM, BL, and ESP ecotypes were less than one, which means that giant reed ecotypes poorly transported Cu from root system (underground part) to shoot system (aboveground part). This could point out that for complete and efficient phytoremediation process, removing the whole plant including its root system is mandatory. However, estimated TF values for all investigated ecotypes increased firstly then declined significantly with increasing Cu concentration above 200 mg kg^{-1} supposing transport channels of roots of giant reed plants were damaged above 200 mg Cu kg^{-1}. Both BL and ESP ecotypes had the highest TF values which were 0.29 and 0.14, respectively, when Cu concentration was 200 mg kg^{-1}, while STM ecotype resulted in highest TF value when Cu concentration was 100 mg kg^{-1} recording 0.41 (Fig. 34). STM plant had the highest value of TF at low Cu concentration, whilst BL and ESP plants at high Cu concentration translocated higher amount of Cu throughout their tissues of shoot part. These results of TF were in consistent with those of Wu Qi et al. (2012).

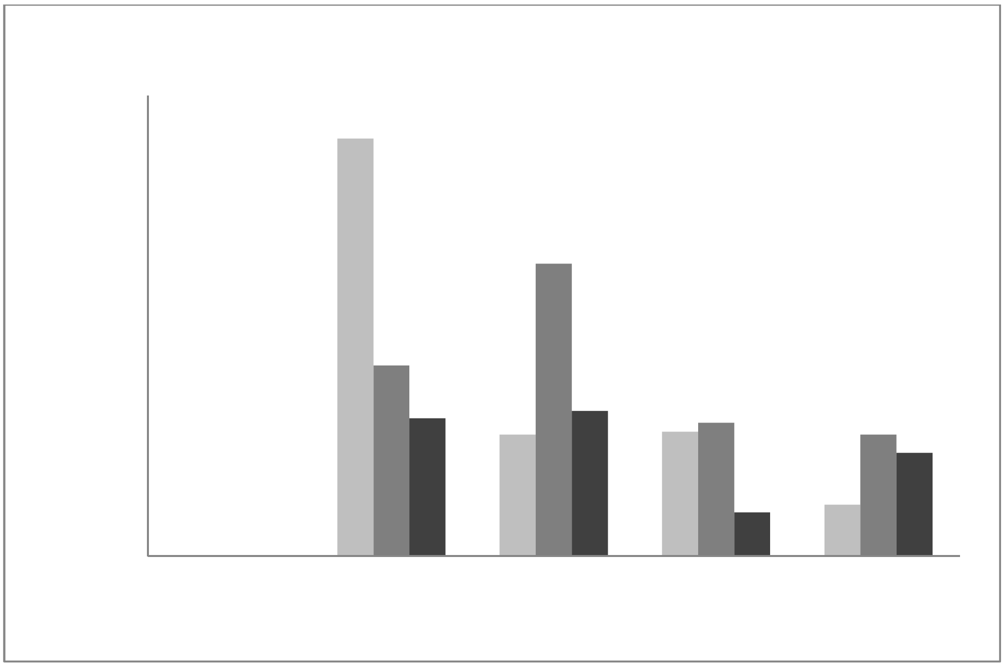


Fig. 34: Transportation factor (TF) for Cu uptake of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

Conversely to TF values, estimated values of BCF were higher than one for all giant reed ecotype used in current study at all treatments of Cu. Increasing Cu concentration resulted in significant differences ($p < 0.05$) for BCF values among investigated ecotypes. The lowest measured BCF value was 2.6 recorded for BL plant when Cu concentration was 400 mg kg^{-1} (Fig. 35). However, calculated BCF values for STM and BL ecotype firstly increased then significantly reduced with increasing Cu concentrations above 200 mg kg^{-1}. On the other hand, ESP plants were less affected negatively with increasing Cu levels in growth medium, where it recorded BCF values of 6.2, 3.4, 5.7 and 5.0 when Cu concentrations ranged from 100 to 400 mg kg^{-1}, respectively. From this data, it became clearly that ESP ecotype had higher capacity to bioconcentrate Cu within its tissues compared to BL and STM ecotypes except when Cu concentration was 200 mg kg^{-1}. Similarly, same results for BCF of giant reed plants grown on increasing Cu levels were reported by Wu Qi et al. (2012).

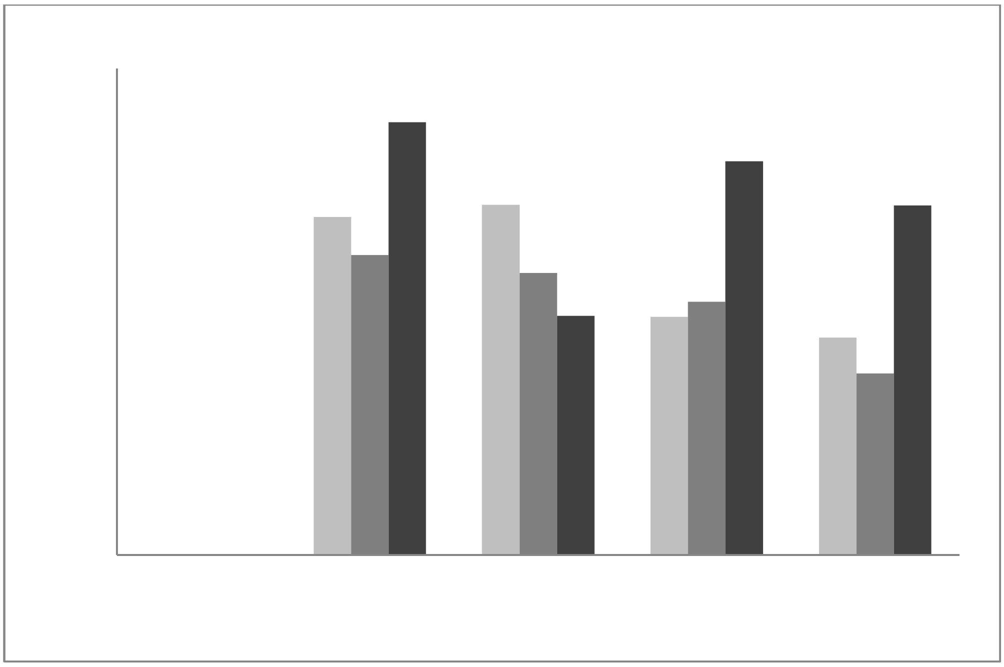


Fig. 35: Bioconcentration factor (BCF) for Cu uptake of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

Substantially, all studied ecotypes had same efficiency towards removing Cu from soil-like growth medium. Values of removal rate (RR) ranged from minimum 0.81 to maximum 0.89 regarding STM, BL, and ESP ecotypes (Fig. 36). However, with increasing Cu concentrations from 100 to 400 mg kg^{-1} no big differences were determined for RR under investigated giant reed ecotypes. Capacity of STM plant for removing declined when Cu increased in soil from 100 to 400 mg kg^{-1}, where RR values were 0.88, 0.82, 0.81, and 0.83, respectively. While, ESP plant possessed gradually reduced values for RR with increasing Cu levels in experimental pots, BL had no clear trend for its RR value where it had RR of 0.89 when Cu concentration was 100 mg kg^{-1} then decreased to reach 0.85 at treatment of 300 mg kg^{-1} but increased again to be 0.89 at highest Cu treatment (400 mg kg^{-1}). These results encourage the recommendation of giant reed ecotypes for Cu phytoremediation purposes, where they proved high efficiency and ability for theoretical removing Cu from Cu-contaminated site with some of 89%.

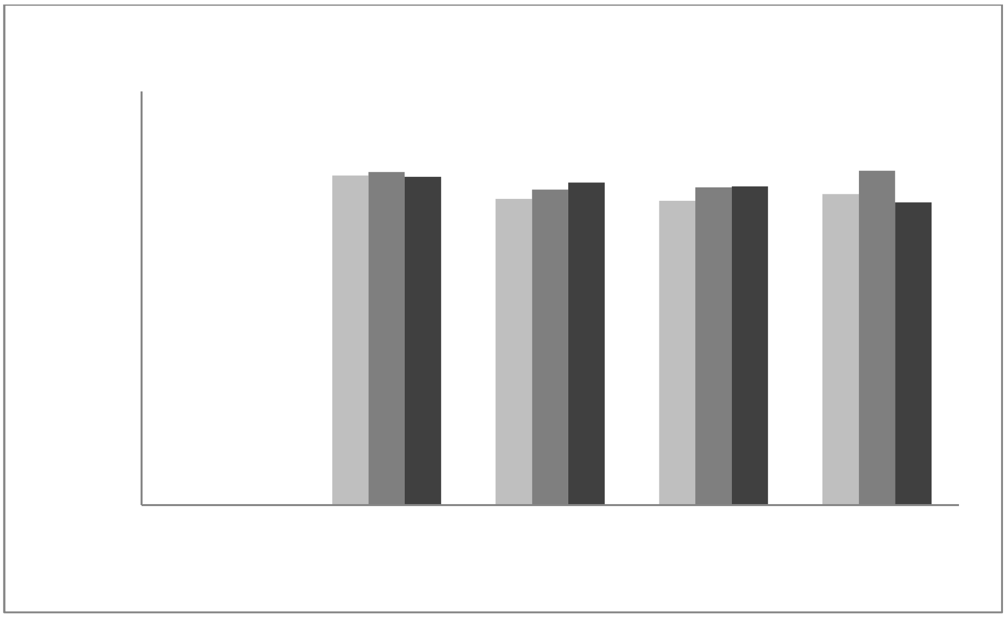


Fig. 36: Removal rate (RR) of Cu by giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

4.2.8. Total soluble protein content of giant reed ecotypes

Total soluble protein content of root system developed giant reed ecotypes grown on increasing Cu concentration under greenhouse conditions is depicted in Fig. 37. Basically, adding Cu to soil-like growth medium enhanced the protein content of root system of all three studied ecotypes of giant reed, where plants grown on increasing Cu concentrations had higher protein contents of root system compared to control plants. When Cu concentration was 400 mg kg^{-1}, protein content of root system of STM, BL, and ESP plant was the highest among all Cu treatments. All three ecotypes had gradual increase of protein content especially ESP ecotype which had the highest protein content (47 g kg^{-1} DM) when Cu oncentration was 400 mg kg^{-1}. Although, there was more stress on giant reed plants of STM, BL, and ESP ecotypes as a consequence for increasing Cu concentration in growth medium, but protein content of root system of all ecotypes increased with increasing Cu levels recording the highest values at highest Cu treatment. This data proved that giant reed ecotypes are tolerant for high Cu concentrations, thus they could introduced as phytoremediation candidates.

Generally, protein content of plant culm of STM, BL, and ESP ecotypes growing on increasing Cu concentrations was similar to protein content of root system.

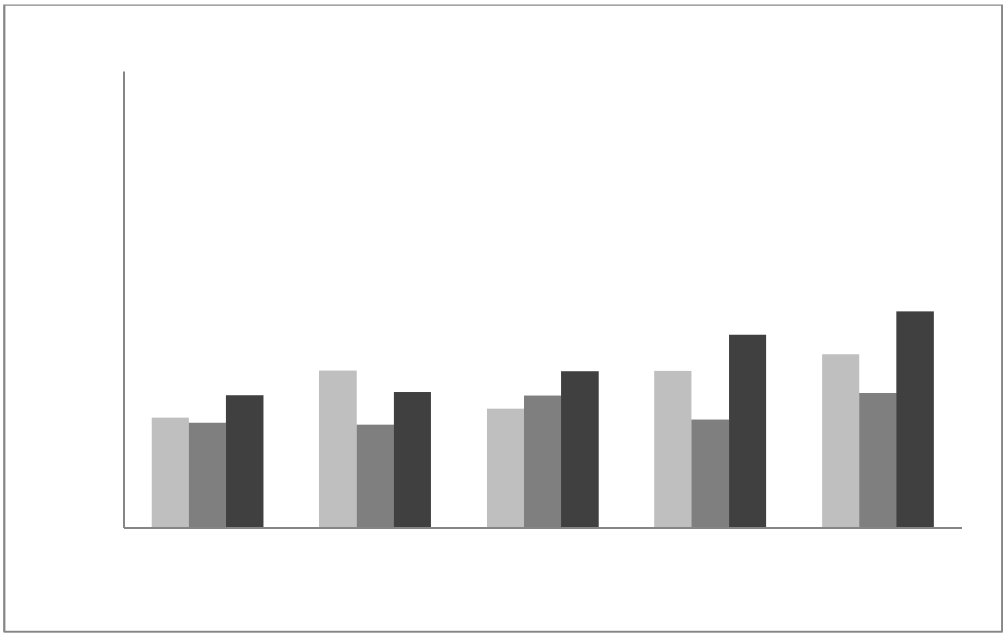


Fig. 37: Total soluble protein content of root system of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations under greenhouse conditions. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

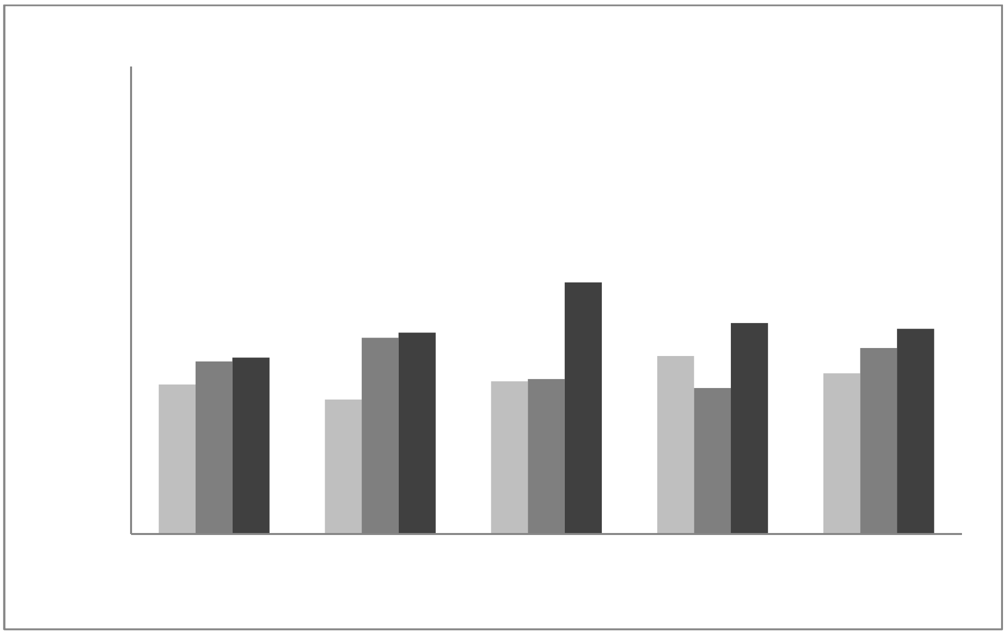


Fig. 38: Total soluble protein content of culm of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations under greenhouse conditions. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

However, all ecotypes recorded an increase for protein content of culm when Cu concentration increased in soil but this increase was lower than that of root system maybe because accumulated Cu within tissues of culm was lower than that measured in root system at same treatment. Both STM and ESP ecotypes had lower values of protein content of culm than root system, while BL plant measured higher content of protein in culm than root system (Fig. 38). The highest measured value of protein content was found in ESP plant in all treatments compared to STM and BL ecotype. Anyway, increasing Cu concentration resulted in higher protein contents of plant culm of studied ecotypes compared to control plant.

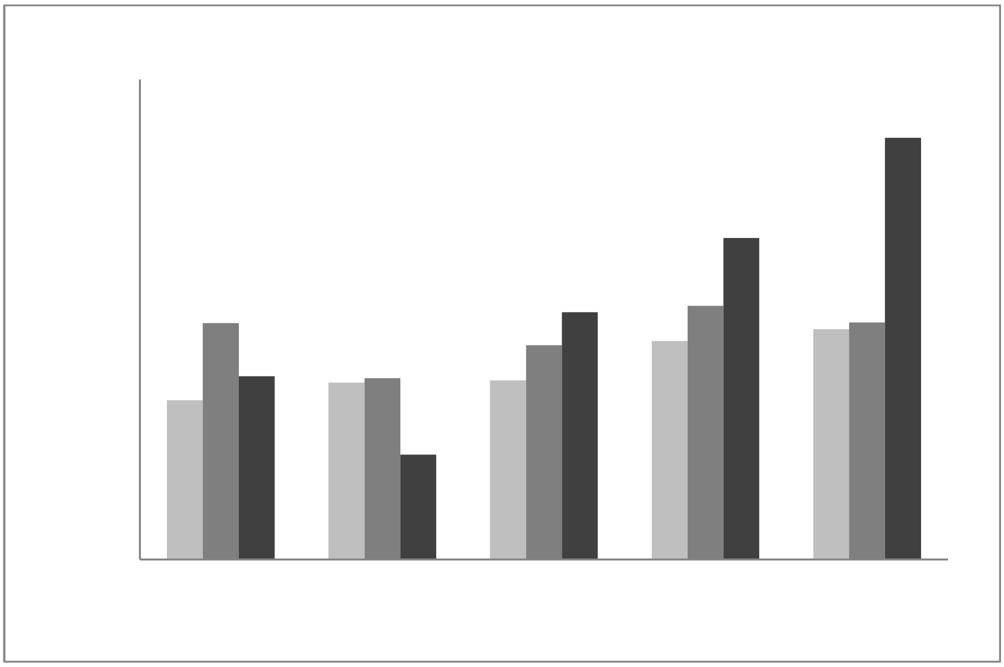


Fig. 39: Total soluble protein content of leaf blade of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations under greenhouse conditions. Different letters over columns show significant differences among groups of treatments according to Duncan's test at $p < 0.05$.

The highest protein content in different plant parts of studied ecotypes was found in leaf blade (Fig. 39). Moreover, STM, BL, and ESP ecotypes had the highest protein contents in their tissues of leaf blade at all treatments compared to protein contents in root systems and culm. Protein content of STM leaf blade regularly increased with increasing Cu concentration significantly, where highest protein content was 48 g kg^{-1} DM when Cu concentration was 400 mg kg^{-1}. Similarly, protein content of ESP leaf blade significantly enhanced with increase of Cu doses reaching highest content (88 g kg^{-1} DM) at treatment of 400 mg kg^{-1}. But there was one exception for ESP ecotype, when Cu concentration was 100 mg kg^{-1} DM protein content was 22 g kg^{-1} DM which was the lowest measured value among all ecotypes at all treatments including control plants. On the other hand, BL ecotype had hesitated response towards increasing Cu concentrations. Firstly protein content of BL leaf blade increased, then declined when Cu concentration was 400 mg kg^{-1}, but protein contents at treatments of 100 and 200 mg Cu kg^{-1} were lower than control plant.

However, these results of protein content of different plant parts of giant reed ecotypes were in contrast to those reported by Zengin and Kirbag (2007). They studied the effect of different levels of Cu (II) chloride on growth dynamics and protein content in sunflower, they documented that reduction of 15–37.5% were measured for total protein content in sunflower seedlings compared to control plant. Exposure of living organisms such as plants to both biotic and/ or abiotic stresses like increasing levels of heavy metals causes reversible and irreversible changes in metabolism. Consequently, total protein content is an important indicator for health of plants (Singh and Tewari, 2003). Some literatures cited increasing Cd concentrations resulted in significant reduction of protein content in *Brassica juncea* L. and root tips of barley seedlings (Singh and Tewari, 2003; Liu et al., 2005). Additionally, other literatures found that protein level declined in many plant species as a consequence for high Cu concentrations (Chen et al., 2001; Singh et al., 2007).

4.2.9. Malondialdehyde content of giant reed ecotypes

Basically, malondialdehyde content (MDA) is usually used as an indicator for lipid peroxidation under abiotic stressors (Shah et al., 2001; Zhang et al., 2005). MDA is product of decomposed unsaturated fatty acids exist in cell membrane. Lipid peroxidation has damaging effects on the cell biomembrane and ultrastructure (Wang, 2005). MDA content of root systems of STM, BL, and ESP ecotypes significantly increased with adding Cu to soil-like growth medium compared to control plant. While, STM and ESP control plants had almost same content of MDA in their root systems, content of MDA of BL ecotype was nearly half of that found for STM and ESP ecotypes (Fig. 40). With applying Cu treatments, MDA content of root system in all ecotypes was closely same; this indicates that Cu treatments had stronger effect on root system of BL plant than STM and ESP plants. However, MDA content of STM root regularly increased with increasing Cu concentrations, where it was 26.3 nmol g^{-1} DM when Cu concentration was 400 mg kg^{-1} compared to 14.2 nmol g^{-1} DM for control plant. Likewise, MDA content of root of ESP plant positively increased with elevating Cu levels, where control plant measured 15.4 nmol g^{-1} DM but plant at treatment of 400 mg Cu kg^{-1} had MDA content of 23.6 nmol g^{-1} DM. Response of BL ecotype for elevated Cu doses was slightly different from STM and ESP ecotypes. MDA content was 7.1 nmol g^{-1} DM for control plant and increased gradually recording 22.6 nmol g^{-1} DM when Cu concentration was 300 mg kg^{-1} then a small decreasing was measured when Cu concentration was 400 mg kg^{-1}. However, ESP ecotype had the highest content of root MDA among all studied ecotypes at all Cu treatments except highest Cu treatment where highest MDA content was measured for STM plant.

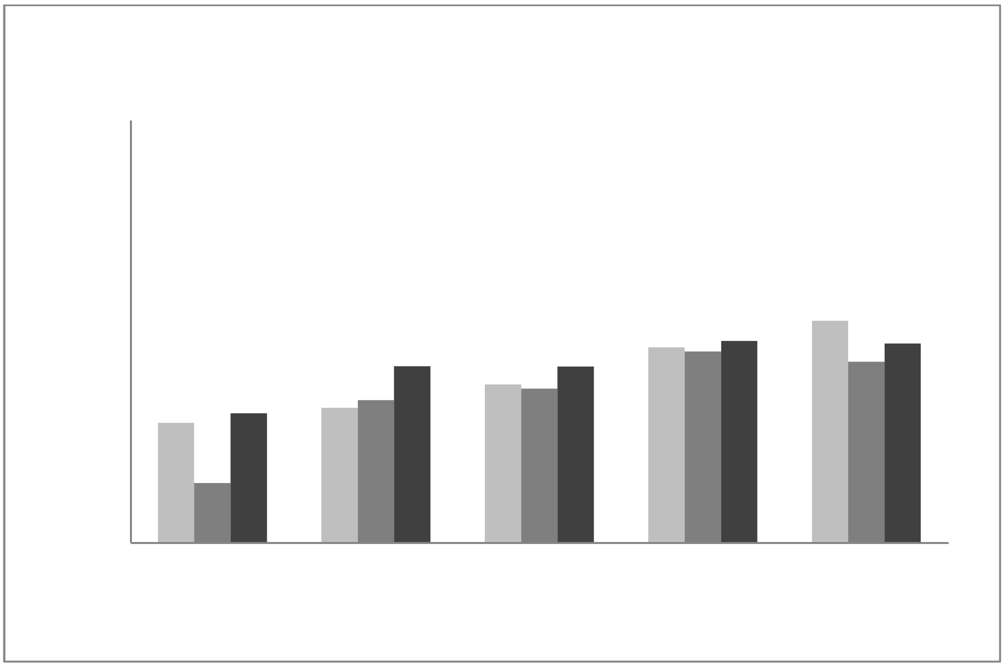


Fig. 40: Malondialdehyde (MDA) content in root system of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over same columns show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

MDA content in the culms of STM, BL, and ESP ecotypes grown under different concentrations of Cu was significantly enhanced versus the control (Fig. 41). At control treatment ESP culm had the highest MDA content compared to STM and BL, it measured almost 2 folds than STM and less than 2 folds for BL. However, when Cu doses increased in soil-like growth medium, MDA content of culm was gradually increased for all studied ecotypes. STM ecotype showed different respond for increasing Cu concentrations than BL and ESP. While the highest MDA content in BL and ESP were found at highest Cu treatment (400 mg kg^{-1}), the highest MDA content of STM culm was measured when Cu concentration was 300 mg kg^{-1}. These results indicated that STM was more resistant to higher Cu concentrations than BL and ESP.

Similarly, MDA content in the leaf blades of STM, BL, and ESP was significantly increased with increasing Cu levels. Leaf blade of control plants of all ecotypes had the lowest MDA content compared to Cu treatments. However, 9.8 nmol g^{-1} DM of MDA were measured in leaf blades of STM control plant but 19.5 nmol g^{-1} DM of MDA was found at the highest Cu treatment. On the other hand, leaf blades of BL had 11.9 and 18.2 nmol g^{-1} DM of MDA at treatments of 0 and 400 mg Cu kg^{-1}, respectively. Leaf blades of STM recorded the highest content of MDA at no Cu treatment achieving 18.7 nmol g^{-1} increased to 24.3 nmol g^{-1} DM when Cu was 400 mg kg^{-1} (Fig. 42).

However, results of MDA contents in various parts of giant reed ecotypes which examined in the present study were in convenience with that reported by Meng et al. (2007). They studied the effects of increasing Cu^{2+} on growth of garlic seedlings and reported that MDA contents in roots exposed to 10^{-4} M and 10^{-3} M Cu^{2+} increased versus the control, indicating that Cu^{2+} indirectly produced superoxide radicals, resulting in increased lipid peroxidative products and oxidative stress in garlic plants. MDA concentrations did not greatly increase during most of the experimental period under 10^{-5} M Cu^{2+} stress. In addition, Zhao et al. (2010) documented that MDA content in *Festuca arundinacea* roots increased significantly in the Cu treatments ($p < 0.05$). For *Lolium perenne*, though, there was no significant difference between Cu treatment and the control except at 90 mg L^{-1}, where there was a great increase in MDA content. The increased MDA content indicates that Cu caused severe oxidative stress by stimulating ROS generation (Dietz et al., 1999). Similar observations have been reported in *Withania somnifera* (Khatun et al., 2008) and oat (Luna et al., 1994).

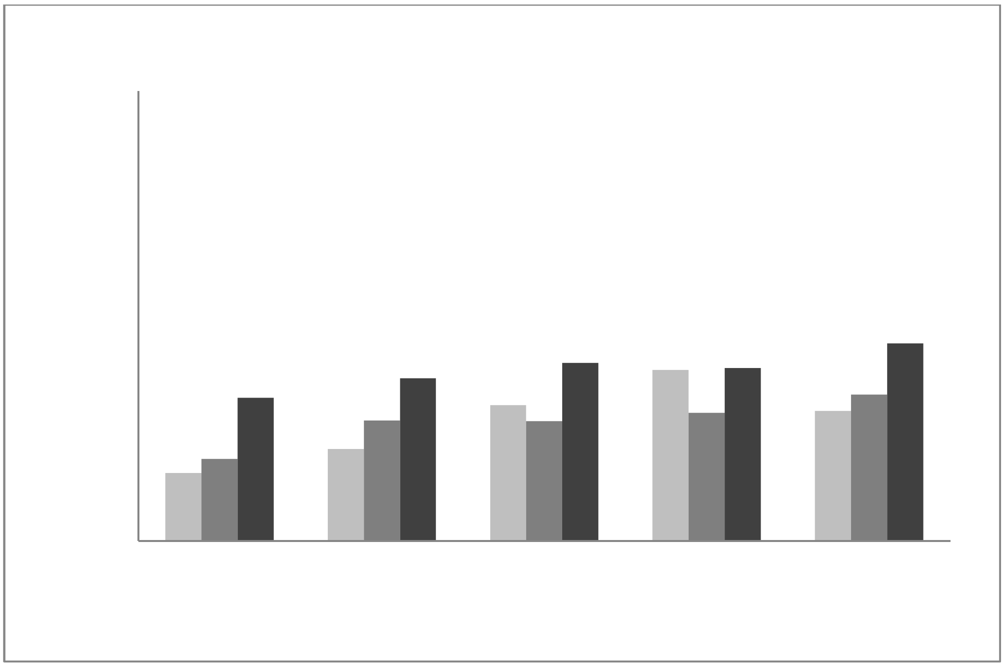


Fig. 41: Malondialdehyde (MDA) content in culm of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over same columns show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

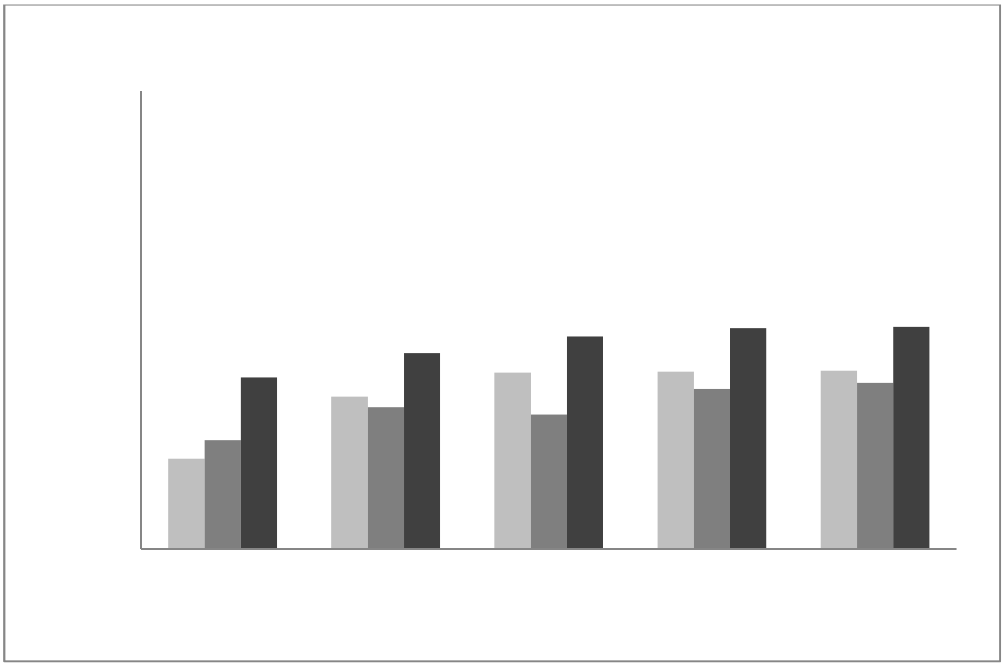


Fig. 42: Malondialdehyde (MDA) content in leaf blade of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters over same columns show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

4.2.10. Chlorophylls *a* and *b* and carotenoids contents of giant reed ecotypes

Photosynthesis is one of the most fundamental and complex metabolic processes closely related to the normal plant growth and development. Environmental factors, such as drought, salinity, heavy metals and/or unfavourable temperatures, considerably can hamper it (Ashraf and Harris, 2013). Chlorophyll *a* and *b* contents and carotenoids of leaves of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations under greenhouse conditions were measured.

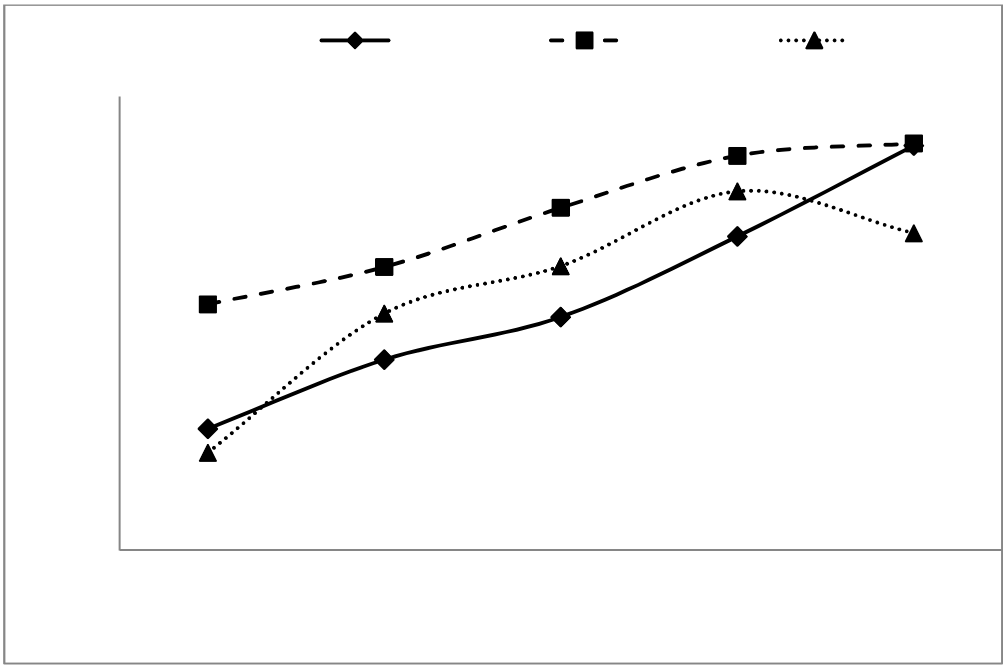


Fig. 43: Content of chlorophyll *a* in leaves of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

Figure 43 displays chl *a* content in leaves of STM, BL, and ESP ecotypes. Clearly, increasing Cu doses had significant effect on chl *a* content in all ecotypes. Leaves of BL plant had the highest content of chl *a* at all Cu treatments compared to STM and ESP plants. However, chl *a* content in leaves of BL plant changed from 32 to 46 mg cm^{-2} when Cu concentration increased from 0 to 400 mg kg^{-1} in soil-like growth medium. Regular increase in chl *a* content was measured in leaves of STM plant when Cu concentration increased in soil-like growth medium up to 400 mg kg^{-1}. Similar data was found for ESP ecotype, where chl *a* content increased with increasing doses of Cu but the highest increase of chl *a* content was recorded when Cu level was 300 mg kg^{-1}. These findings illustrated that all giant reed ecotypes under investigation are tolerant to high Cu concentrations, therefore they could act as phytoremediation candidates for Cu-contaminated sites.

Chl *b* content in leaves of STM, BL, and ESP ecotypes was significantly increased with increasing Cu concentrations in soil-like growth medium. When Cu doses increased in soil-like growth medium up to 300 mg kg^{-1}, linear increase in chl *b* content in leaves of STM plant was measured. But an exponential increase was found for chl *b* content in leaves of STM when Cu concentration increased up to 400 mg kg^{-1} (Fig. 44). BL and ESP plants responded to elevated Cu concentrations positively. Content of chl *b* in leaves of BL and ESP plants firstly increased with increasing Cu concentrations up to 300 mg kg^{-1}, then slightly decreased with increasing Cu to 400 mg kg^{-1}. The highest chl *b* content in leaves of BL ecotype was 63.2 mg cm^{-2} when Cu was 300 mg kg^{-1}. In contrast to BL ecotype, chl *b* content in ESP leaves exponentially increased when Cu was 100 mg kg^{-1} compared to control, then chl *b* content increased gradually above that found in control plant with increasing Cu levels up to 300 mg kg^{-1}. But with increasing Cu to 400 mg kg^{-1} chl *b* content slightly decreased (Fig. 44). In addition, the highest content of chl *b* in leaves of ESP plant was detected at treatment of 300 mg Cu kg^{-1} as found in BL ecotype.

Carotenoids contents (µg cm^{-2}) in leaves of growing giant reed ecotypes on elevated Cu concentrations are depicted in Fig. 45. Positively increasing Cu levels enhanced carotenoid contents in leaves of studied giant reed ecotype. Significant differences were measured for carotenoid contents with elevating Cu doses. Content of carotenoid in leaves of both BL and ESP plants steadily induced with increasing Cu concentrations up to 300 mg kg^{-1} then decreased when Cu concentration was 400 mg kg^{-1}. In leaves of STM plant carotenoid content linearly increased with increasing Cu concentrations, where highest content of carotenoid was 19 µg cm^{-2} when Cu concentration was 400 mg kg^{-1}. However, treatments of 100 and 200 mg Cu kg^{-1} did not resulted in siginificant differences of carotenoids content.

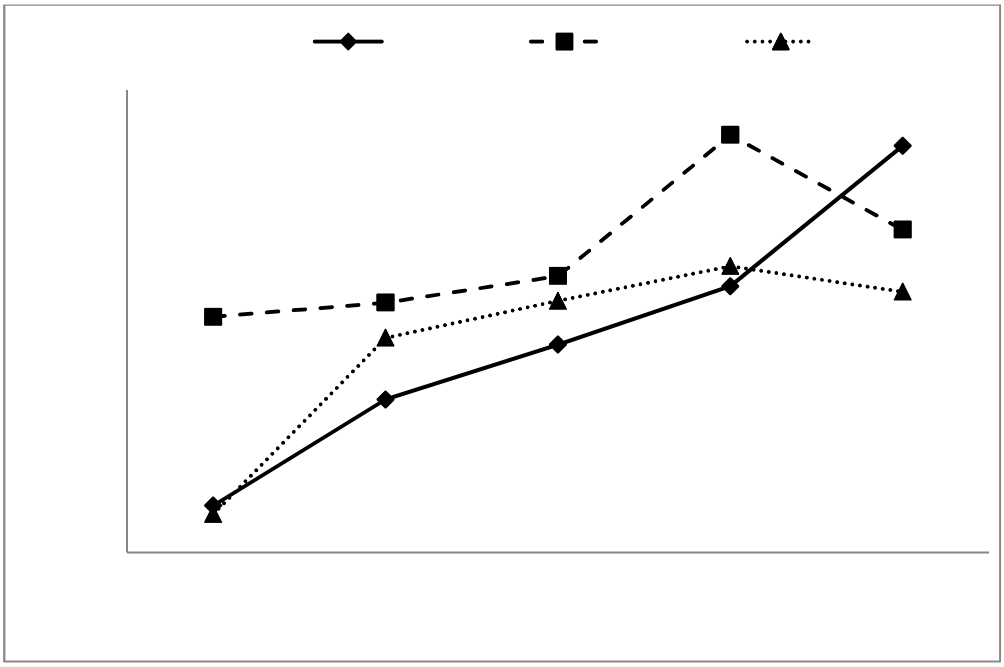


Fig. 44: Content of chlorophyll *b* in leaves of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

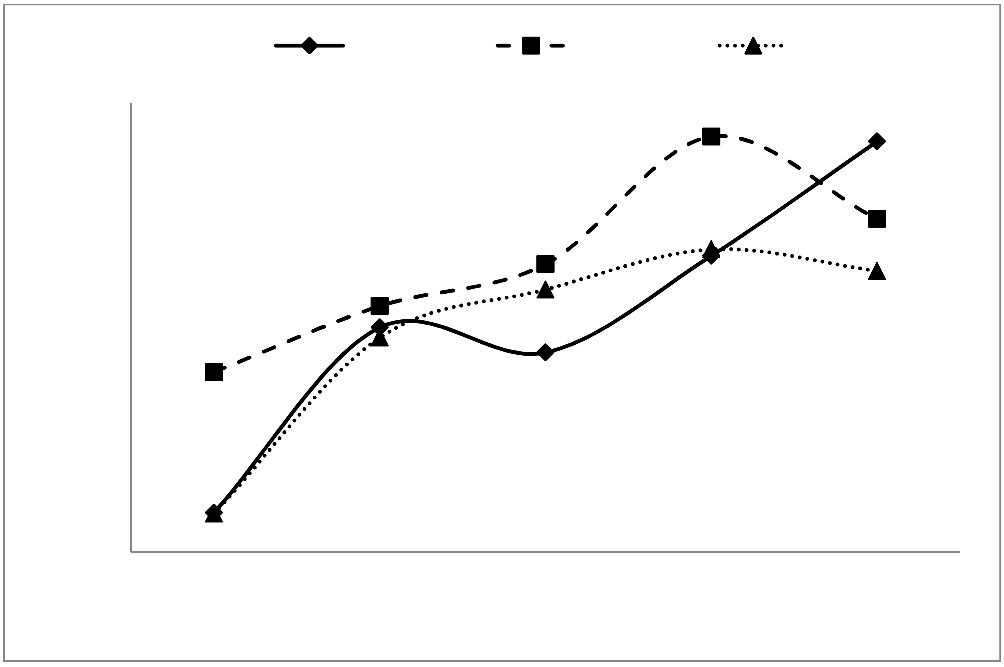


Fig. 45: Content of carotenoids in leaves of giant reed ecotypes (STM, BL, and ESP) grown on elevated Cu concentrations. Different letters show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

The results of chlorophyll *a* and *b* contents in leaves of giant reed ecotypes were in contrast to that reported by Dey et al. (2014). They studied the effect of Cu concentrations on growth and chlorophyll pigments of tea plants. They documented that there was a significant decrease in the total chlorophyll pigment in both the treated cultivars of tea plants up to 600 μM Cu with respect to control. Trace elements have deleterious effects on plant metabolism, but some metals such as Cu, Fe, and Mn are essential for the photosynthesis in trace amount but in high concentration they have toxic effects on photosynthesis (Arun et al., 2005). Toxic levels of trace elements like Cu cause degradation of photosynthesis enzymes which result in the decline of chlorophyll content (Thapar et al., 2008).

At the physiological level, the measurement of photosynthetic efficiency (Fv/Fm) is useful and effective parameters to assess the photosynthetic status of plants under heavy metals stress conditions. Table 8 showed values of Fv/Fm measured in leaves of STM, BL, and ESP ecotypes after growing on elevated Cu concentrations. Greenhouse experiment has been started in mid of July 2013 and one and half months later (September) Fv/Fm was measured and after one month (October) it was measured again. However, with respect to control plants no significant differences were measured at Cu treatments for all investigated ecotypes of giant reed. In September, the highest values of Fv/Fm of STM, BL, and ESP ecotypes were found at the highest Cu treatment (400 mg kg^{-1}). Values of Fv/Fm of STM plant ranged from 0.80 to 0.83 at treatments of 0 to 400 mg Cu kg^{-1}. While, BL plants had values changed from 0.81 to 0.84, ESP plants recorded values of 0.81 to 0.81 when Cu concentrations changed from 0 to 400 mg kg^{-1}. In general, values of Fv/Fm that measured in October were higher than that measured in September within all ecotypes, but this increase was not significant. As a consequence for non-siginificant values of Fv/Fm that measured under different Cu treatments with respect to control, it could be concluded that giant reed ecotypes did not negatively affected with high Cu concentrations, but have been enhanced therefore they showed tolerant characteristics for elevated Cu levels. Cu-induced chlorosis can result from the inhibition of pigment accumulation and decrease in the chlorophyll integration into photosystems. As a whole it causes damages in the structure and function of the chlorophyll (Kupper et al.; 2003). Net photosynthesis efficiency did not significantly ($p < 0.05$) influenced when giant reed plants irrigated with increasing cconcentartions of

Cd and Ni for 2 years in pot experiment under open field conditions (Papazoglou et al., 2005).

Table 8: Maximum quantum efficiency of PSII (Fv/Fm) of dark adapted leaves of giant reed ecotypes treated with elevated Cu concentrations

Cu doses	September			October		
(mg kg^{-1})	*STM*	*BL*	*ESP*	*STM*	*BL*	*ESP*
0	0.80cde	0.81abcd	0.81bcde	0.83ab	0.83ab	0.83ab
100	0.83ab	0.83a	0.80de	0.82abc	0.83ab	0.79d
200	0.82abcd	0.82abcd	0.81bcde	0.83ab	0.83ab	0.81bcd
300	0.83ab	0.82abcd	0.79e	0.83ab	0.84a	0.80cd
400	0.83abc	0.84a	0.81bcde	0.83a	0.84a	0.82ab

In columns different letters show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

4.2.11. Impact of Cu on efficiency of CO_2 assimilation

Atmospheric CO_2 assimilation is performed in Calvin-Benson cycle in the chloroplast stroma involving a series of enzymatic reactions. CO_2 assimilation (µmol m^{-2} sec^{-1}) of giant reed ecotypes was measured twice, firstly at September (after one and half months from transplantation) and second time was in October. In September, Cu reflected significant toxic impacts on values of CO_2 assimilation in all ecotypes, specially STM and ESP plants. However, Cu treatments resulted in lower values of CO_2 assimilation for all ecotypes. Treatment of 300 mg Cu kg^{-1} caused the greatest reduction in CO_2 assimilation in STM and ESP plants recording 1.96 and 0.35 µmol m^{-2} sec^{-1}, respectively (Fig. 46). For BL ecotype, while 2.48 µmol m^{-2} sec^{-1} was measured when Cu level was 100 mg kg^{-1}, the higher Cu concentrations enhanced CO_2 assimilation. Gradual increase was noticed for CO_2 assimilation in BL plants when Cu concentrations increased above 100 mg kg^{-1}. In second measuring of CO_2 assimilation in October, Cu treatments showed positive effects on CO_2 assimilation, where all treatments of all ecotypes had higher values of CO_2 assimilation versus control plants (Fig. 47).

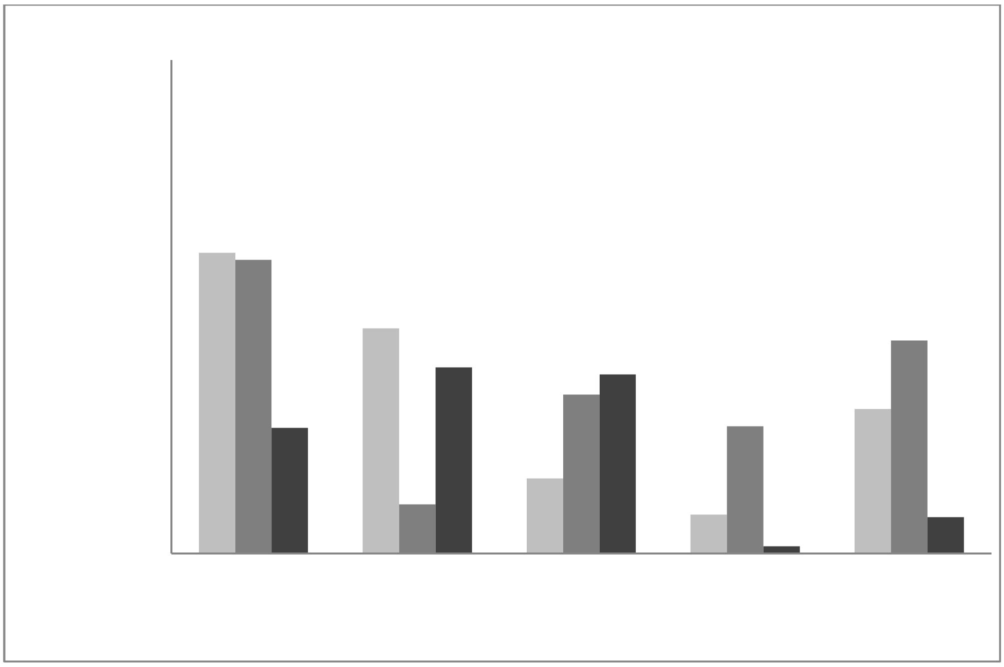


Fig. 46: Alteration in net CO_2 assimilation rate in leaves of giant reed ecotypes treated with elevated Cu concentrations (in September). In columns different letters show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

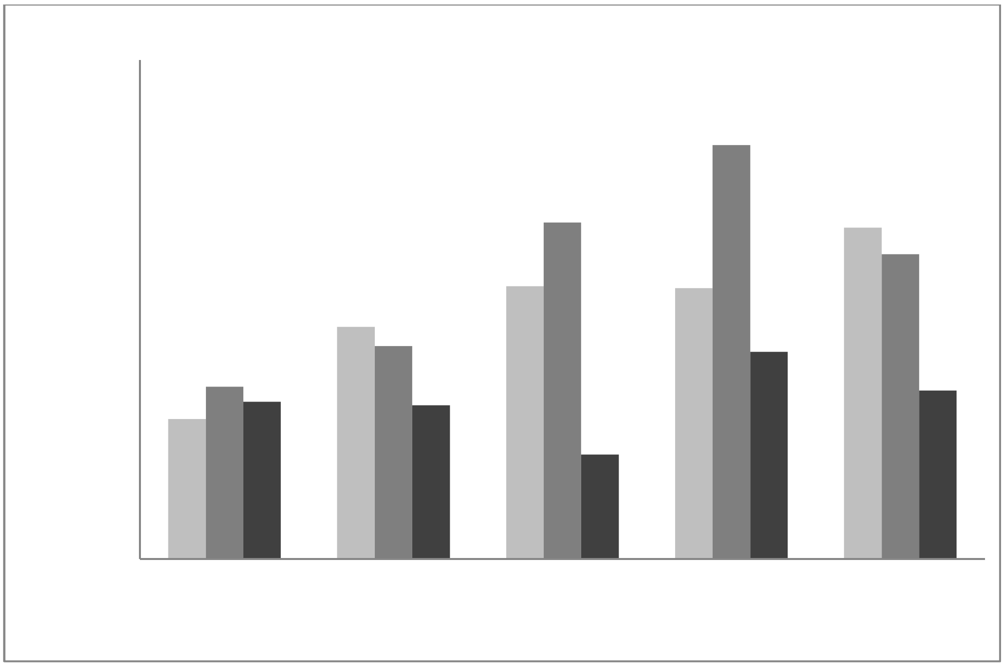


Fig. 47: Alteration in net CO_2 assimilation rate in leaves of giant reed ecotypes treated with elevated Cu concentrations (in October). In columns different letters show significant differences among each group of treatments according to Duncan's test at $p < 0.05$.

STM plants refelected steadily increase in CO_2 assimilation with increasing Cu levels in soil-like growth medium, where highest value 16.62 µmol m^{-2} sec^{-1} was measured at treatment of 400 mg kg^{-1}. Concerning BL ecotype, firstly CO_2 assimilation was sharply increased to 20.75 µmol m^{-2} sec^{-1} when Cu concentrations increased up to 300 mg kg^{-1} then decreased to be 15.28 µmol m^{-2} sec^{-1} when Cu was 400 mg kg^{-1}. On the other hand, ESP plant had the lowest values of CO_2 assimilation compared to STM and BL ecotypes. Both of treatments of 100 and 200 mg Cu kg^{-1} showed toxic impacts on CO_2 assimilation values, where they resulted in lowest measured values of CO_2 assimilation among all treatments. However, Cu concentrations of 300 and 400 mg kg^{-1} were totally opposite to lower Cu concentrations, where they increased the CO_2 assimilation achieving 10.38 and 8.44 µmol m^{-2} sec^{-1}, respectively. Finally, from all the mentioned above results, it could be summarized that all giant reed ecotypes used in this present study (STM, BL, and ESP) showed high resistant to high Cu concentrations up to 400 mg kg^{-1}. Although, data of transportation and bioconcentration factors are lower than reported with metals hyperaccumulator candidates, but the high biomass yield of these ecotypes and their great tolerance for high Cu levels as well as their ability to grow under unfavourable growth conditions strongly recommend the using of these ecotypes for phytoremediation purposes of Cu-contaminated environments up to 400 mg kg^{-1}.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. *In vitro* experiments

5.1.1. Growing giant reed ecotypes on liquid medium

- Giant reed ecotypes (BL and 20SZ) which are used in the present study showed great tolerance to elevated Cu concentrations up to 26.8 mg L^{-1} in artificially Cu-contaminated water.

- No Cu toxicity symptoms were reported on shoot and root parts of both ecotypes up to 10 mg Cu L^{-1}, but some reduction in root systems were noticed at treatment of 26.8 mg Cu L^{-1}.
- Both giant reed ecotypes showed capacity to uptake Cu from artificially contaminated water bodies, where the removal rates ranged between 96.6 to 100%.
- Almost 50% from total uptake Cu content was found in root system of both ecotypes.
- Transportation and bioconcentration factors had values higher than 1 especially whec Cu concentration was higher than 5 mg L^{-1}.
- Both ecotypes showed considerable and significant efficiency to clean Cu-contaminated water bodies up to 26.8 mg L^{-1}. So, it is a positive indicator of their potential capacity to serve as a phytoremediation plant together with the existence of nontoxic symptoms on the plants. These results emphasized that both ecotypes being nonfood crop, might act as good phytoremediation candidates for Cu-contaminated water bodies.

5.1.2. Growing giant reed ecotypes on solidified medium

- BL and 20SZ giant reed ecotypes grown on solid medium containing Cu up to 26.8 mg L^{-1} without any noticeable symptoms of Cu toxicity.
- Dry mass of plants of both ecotypes was significantly increased at the highest Cu treatment compared to control.
- Although the BL ecotype had higher capacity to uptake Cu than 20SZ, the dry mass, as well as shoot length, of BL was higher than that of 20SZ.

- Values of bioconcentration and transportation factors were higher in the BL than in the 20SZ ecotype.
- Almost 45% of total Cu content within the whole plant was found in plant root of both ecotypes.

These findings encourage the use of giant reed plant for biomass production in Cu-contaminated soil during phytoremediation. Utilization of biomass plants for phytoremediation purposes will achieve an additional benefit by producing a significant biomass production during remediation Cu-contaminated soils.

5.2. *Ex vitro* experiment

- STM, BL, and ESP ecotypes of giant reed which derived by somatic embryogenesis were normally grown on elevated Cu concentrations up to 400 mg kg^{-1}.
- No foliar symptoms for Cu toxicity were seen on aboveground part (shoot) of any ecotype.
- Root system of all ecotypes was significantly reduced at the highest Cu concentration (400 mg kg^{-1}).
- Shoot length of all ecotypes was significantly enhanced with increasing Cu levels in soil-like growth medium.
- Dry mass of root system of giant reed ecotypes was slightly decreased with inceasing Cu doses with respect to control plant, while dry mass of culm and leaf blade was significantly enhanced when Cu concentrations increased in soil-like growth medium. All three ecotypes showed same efficiencies for uptake Cu, where they removed 82.8 to 88.0 % from total Cu under different Cu treatments.
- Most of removed Cu was accumulated in root system of giant reed ecotypes.
- At higher Cu concentrations above 100 mg kg^{-1}, BL plant had higher transportation factor (TF) than STM and ESP, but all values of TF were less than 1.
- Bioconcentration factor (BCF) values were higher than reference value of 1, and ESP recorded the higher values with respect to STM and BL plants.
- Photosynthesis pigments such as chlorophylls *a* and *b* as well as carotenoids content were significantly enchanced with increasing Cu levels.

- Total protein contents in different plant parts of all ecotypes were induced significantly when Cu levels increased in soil-like growth medium.
- Increasing Cu concentrations negatively affected malondialdehyde (MDA) content in all plant parts of STM, BL, and ESP ecotypes, where higher MDA contents were measured under Cu treatments versus control plants.

From all the metioned data above, it could be concluded that giant reed ecotypes showed considerable ability and capacity to grow on elevated Cu concentrations. Also they have great potential for removing Cu from Cu-contaminated soil-like growth medium. Therefore, it could be recommended that giant reed ecotypes can be employed as phytoremediation candidate as well as producing significane biomass production during remediation process.

6. NEW SCIENTIFIC RESULTS

- Using giant reed ecotypes for decontaminating Cu-contaminated water bodies due to its ability to translocate the Cu from roots to shoots.

- Industerial wastewater polluted with Cu can be restored by giant reed ecotypes, where they showed significant ability to remove Cu from wastewater reached some of 92 to 99%.

- In soil-like environemnts which are contaminated with high Cu levels up to 400 mg kg^{-1}, giant reed ecotypes can remediate it with efficiencies ranged between 82.6 to 88.0%.

- Photosynthesis pigments positively enhanced by elevated Cu concentrations; this indicates that studied giant reed ecotypes are tolerant to high Cu levels up to 400 mg kg^{-1}.

- High Cu exposures did not negatively affect the biomass production of giant reed plants; therefore considerable biomass production is expected for giant reed ecotypes during the phytoremediation process as an additional benefit.

7. SCIENTIFIC RESULTS UTILIZABLE IN THE PRACTICE

- Giant reed plants showed considerable effects to remediate the Cu-contaminated streams
- The possibility of using giant reed plants in Cu-contaminated soil-like growth medium, since it showed high tolerance for growing on increasing Cu levels.
- Giant reed is considered as good candiate for restoring and remediating Cu-contaminated environments, where it showed great capacity and efficiency for uptake Cu from contaminated soil-like growth medium.
- Using giant reed as bioenergy crop during the phytoremediation process, where it can produce significant biomass production especially on Cu-contaminated sites.

8. SUMMARY

An *in vitro* experiment was carried out to evaluate the phytoremediation potentials of two somatic embryo-derived ecotypes of giant reed (*Arundo donax*) — BL (American ecotype) and 20SZ (Hungarian ecotype) — of copper (Cu) from artificially contaminated water. The two ecotypes were grown under sterile conditions in tubes containing a nutrient solution supplied with increasing doses of Cu (0, 1, 2, 3, 5, 10, and 26.8 mg Cu L^{-1}) for 6 weeks. TF, BCF, and RR were estimated. Both ecotypes showed no toxicity symptoms after growing on elevated Cu concentrations up to 10 mg L^{-1} treatment, only BL ecotype at 26.8 mg L^{-1} recorded short root and culm length with yellowish color of leaves. Plants tend to accumulate Cu in their root tissues with small transportation rates to the shoots at low Cu concentrations. Therefore, alterations in root growth and morphology occur before any toxic symptoms appear on shoots. For both ecotypes BL and 20SZ, more than 59 and 58% of total Cu in plant tissues was found in root only. Roots are especially vulnerable to Cu toxicity. Moreover, both ecotypes showed high Cu removal efficiency from aqueous solution. However, Cu removal rate ranged between 96.6 to 98.8 % for BL ecotype and 97 to 100 % for 20SZ ecotype. Data illustrated that both BL and 20SZ ecotypes may be employed to treat Cu-contaminated water bodies up to 26.8 mg L^{-1}.

The second experiment aimed to investigate the possible utilization of Cu-contaminated soils for biomass production during the phytoremediation process using two ecotypes of giant reed. Experiment was carried out using BL and 20SZ grown on a series of Cu concentrations, i.e., 1, 2, 3, 5, 10, and 26.8 mg Cu L^{-1}. Neither ecotype showed any noticeable foliar symptoms of Cu toxicity at concentrations tested up to 10 mg L^{-1}. Dry mass of plants of both ecotypes was significantly increased at the highest Cu treatment compared to control. Although the BL ecotype had higher capacity to uptake Cu than 20SZ, the dry mass, as well as shoot length, of BL was higher than that of 20SZ. Values of bio-concentration and transportation factors were higher in the BL than in the 20SZ ecotype. Almost 45% of total Cu content within the whole plant was found in plant root of both ecotypes. These results are encouraging the use giant reed for biomass production in Cu-contaminated soils alongside with the phytoremediation process.

Despite that different plant parts of BL ecotype accumulated more Cu than the 20SZ ecotype; dry mass of plant parts of BL ecotype was higher than 20SZ ecotype especially at the highest Cu concentration. However, dry mass of root, culm and leaf were 0.16, 0.14 and 0.31 g plant^{-1} for BL ecotype and 0.15, 0.06 and 0.09 g plant^{-1}, respectively, at treatment of 26.8 mg L^{-1}. These findings might indicate that BL ecotype can adapt easily and faster with high Cu concentration than 20SZ within this period of plant growth. Moreover, BL ecotype may need more Cu than 20SZ ecotype for better growth since Cu is an essential micronutrient. Nevertheless, this behavior of BL ecotype towards Cu is not well understood, where in high Cu concentrations some Cu toxicity symptoms were noticed. At the treatments of 10 and 26.8 mg L^{-1} of Cu, shoot length of BL plant was 9.0 cm in both treatments while for 20SZ plant was 14.5 and 6.5 cm, respectively. Though, 20SZ plants accumulated Cu in low concentrations at the highest Cu treatment but its shoot length was negatively affected by increasing Cu compared to BL plants that had longer shoot at same treatment. On the other hand, the roots system of BL plants was severely affected by Cu.

Furthermore, at the highest Cu concentration the length of BL root was shorter (2.5 cm) than the root of 20SZ plant. This could be due to root Cu content in both ecotypes, where in BL root the Cu content was almost 2 times more than in 20SZ root. The dry mass of BL plant was higher than dry mass of 20SZ plant, although higher contents of Cu were measured in BL than in 20SZ. Both ecotypes showed the same tendency towards Cu uptake from the growth medium in low Cu treatments up to 5 mg L^{-1}. Basically, with increasing Cu in growth medium the Cu content in different plant parts increased as well. But in higher Cu concentrations BL ecotype was able to take up significantly much Cu in its tissues of the root, culm or leaf than 20SZ ecotype. The Cu content in different plant parts of BL ecotype was in the following order: culm < leaf < root culm, while it was in the following order: leaf < culm < root for 20SZ ecotype. However, BL ecotype showed possible utility as a candidate for Cu phytoremediation purposes as opposed to the 20SZ ecotype. However, 41.4% of total Cu in BL plant was accumulated in the root, and 23.3% was in leaf while culm of the plant accumulated 21.0%. On the other hand, root of 20SZ plant contained 45.0% of total Cu content within the plant tissues and 21.7% was in leaf but 18.9% was found in culm of the plant. These results could boost using Cu-contaminated lands for biomass production during phytoremediation, where no high Cu content will be in the shoot part avoiding causing contamination for biomass with Cu. For phytoextraction to work, the plant must have tolerance to the contaminant as well as sufficient uptake and concentration in the harvestable aboveground portion (culm and leaves). The BL ecotype does have the tolerance, so it can be used for biomass production on a contaminated soil. Although its BCF does not qualify it as an accumulator of Cu, the plant's high biomass yield per hectare will still result in a considerable amount of Cu removal; i.e. at an annual yield of 40 dry tons per hectare, a harvest of BL would remove 40.73 mg kg^{-1} amount of Cu.

The pot experiment using soil-like growth medium (sand) under greenhouse conditions – from July to October 2013 – had been started with 2 seedlings per pot of – STM (Hungarian), BL (American), and ESP (Spanish) – giant reed ecotypes. With aim to study the effect of Cu on the survival of plants, number of surviving mother plants is recorded at the end of experiment period. All ecotypes showed considerable tolerance for high Cu concentrations, where elevated Cu levels did not negatively influence the number of mother plants that started the experiment. On the other hand, number of new tillers per plant was positively affected by increasing Cu doses especially for STM and BL ecotypes. While, 6 new tillers per plant were recorded under treatment of 200 and 300 mg kg^{-1} for STM and BL ecotypes, respectively, 4.8 new tillers were noticed for ESP ecotype at no supplied Cu. Length of new tillers was significantly increased with increasing Cu for all ecotypes compared to control. There was consistency in data of number of leaves of new tillers with data of mother plants. Moreover, adding Cu to soil-like growth medium induced the number of leaves of all three ecotypes compared to control plants. High Cu concentration i.e., 400 mg kg^{-1}, significantly reduced length of internodes of mother plants of STM and BL ecotypes compared to control plant. But ESP ecotype was less negatively affected with high Cu treatment, where the longest internode was noticed at treatment of 200 mg Cu kg^{-1} compared to control. At harvest of experiment, data of shoot length showed that plants of STM ecotype were more resistant to increasing Cu levels added to soil-like growth medium than BL and ESP ecotypes. Moreover, all Cu concentrations induced the shoot length of STM plants, where values of shoot length at all treatments of Cu were higher than that of control plants. In contrast to STM and BL ecotypes, plants of ESP ecotype were sensitive to high Cu levels in soil-like growth medium. Increasing Cu doses over 100 mg kg^{-1} significantly reduced shoot length of ESP plants against control treatment.

On the other hand, symptoms of high Cu concentrations were clearly noticed on root system of all giant reed ecotypes. In general, with increasing Cu levels in soil-like growth medium significantly root length reduced for all ecotypes, except STM root system at treatment of 100 mg Cu kg^{-1}. Results of shoot volume of STM plants were in harmony with results of its shoot length. All Cu treatments induced the growth of STM plants and this reflected on its shoot volume. Values of shoot volumes under Cu treatments were higher than that of control plants except treatment of 200 mg Cu kg^{-1}. Only BL plants had tendency for its root volume with increasing Cu levels in soil but both STM and ESP ecotypes recorded hesitated values for their root volumes. With increasing Cu doses volume of root system of BL ecotype significantly decreased. Root system of studied giant reed ecotypes was severely affected by increased Cu doses in soil-like growth medium, but such effects did not record on shoot system. Consequently, wet masses of root system of STM, BL, and ESP ecotypes in the same way decreased with increasing Cu levels. Wet masses of root systems of STM, BL, and ESP ecotypes were obviously reduced with increasing Cu in soil-like growth medium up to 300 mg kg^{-1}. Although, adding Cu to soil-like growth medium by increasing concentrations up to 400 mg kg^{-1}, which is above the normal levels of Cu in soils, no effects on wet masses of culm of all three ecotypes were noticed. In the same way with wet masses of plant culm, results of leaf blade wet mass of ESP, BL, and ESP ecotypes were. In general, all three ecotypes had no clear tendency for dry mass of their root system towards increasing Cu levels in soil used for experiment. However, BL plant showed negative response towards increasing Cu doses, where with increase added Cu the dry mass of root system gradually reduced till treatment of 300 mg kg^{-1} but increased again at 400 mg Cu kg^{-1}. In contrast to dry mass of root system of studied giant reed ecotypes, dry mass of culm was positively affected with increasing Cu doses. All ecotypes had higher values of dry mass of culm compared to control plants. Whilst, STM plants had lowest dry mass of leaf blade among all giant reed ecotypes, in higher Cu treatments above 200 mg kg^{-1} it had the highest dry mass compared to BL and ESP ecotypes.

Significant differences of Cu contents of mother plant of all three studied ecotypes (STM, BL, and ESP) were found with increasing Cu in soil-like growth medium. Mother plant of STM ecotype had the highest content of Cu in its shoot parts compared to other two ecotypes under treatments of 100 and 300 mg Cu kg^{-1}, respectively. However, the highest Cu content in shoot part of mother plant of all ecotypes was 121 µg g^{-1} and found in STM plant at treatment 300 mg kg^{-1}. Cu content in root system of investigated STM, BL, and ESP ecotypes of giant reed significantly increased with increasing Cu concentrations in experimental pots. However, all ecotypes accumulated increasing amounts of Cu within their root systems with increasing Cu level in treatments, where Cu content in root system at treatment of 400 mg Cu kg^{-1} was 3 folds more than Cu content in root system of studied ecotypes at treatment of 100 mg Cu kg^{-1}. Cu content of culm of ESP plant was directly proportional to Cu doses in soil-like growth medium. As Cu increased in the treatments, culm of ESP plant concentrated more Cu in its tissues ranging from 43 to 101 µg g^{-1} at treatments of 100 to 400 mg Cu kg^{-1}, respectively. However, the highest content of Cu (130 µg g^{-1}) among all ecotypes was recorded within culm of BL ecotype at treatment of 200 mg kg^{-1}. Basically, increasing Cu levels did not substantially increase the contents of Cu in leaf blade of all investigated ecotypes, except ESP plant which had the highest recorded Cu content (84 µg g^{-1}) at highest Cu treatment, i.e., 400 mg kg^{-1}. Effectively, adding Cu to soil-like growth medium as increasing doses resulted in increasing the concentration of available Cu in soil after harvesting of giant reed ecotypes. Basically, increasing available Cu concentrations were measured in pots after experiment under STM, BL, and ESP ecotypes. Substantially, increasing Cu concentrations in soil-like growth medium resulted in significant increase of total concentrations of Cu in all pots under all ecotypes. Effect of giant reed ecotypes on total Cu concentration was clearly noticed. However, no significant differences for total Cu concentration were measured at treatments of 100 and 200 mg Cu kg^{-1}, but with increasing Cu in treatments exponential increase was found.

Basically, calculated values of transportation factor (TF) significantly affected with increasing Cu concentrations in soil-like growth medium. All TF values for STM, BL, and ESP ecotypes were less than one, which means that giant reed ecotypes poorly transported Cu from root system (underground part) to shoot system (aboveground part). This could point out that for complete and efficient phytoremediation process, removing the whole plant including its root system is mandatory. However, estimated TF values for all investigated ecotypes increased firstly then declined significantly with increasing Cu concentration above 200 mg kg^{-1}. Conversely to TF values, estimated values of bioconcentration factor (BCF) were higher than one for all giant reed ecotype used in current study at all treatments of Cu. Increasing Cu concentration resulted in significant differences for BF values among investigated ecotypes. Substantially, all studied ecotypes had same efficiency towards removing Cu from soil. Values of removal rate (RR) ranged from minimum 0.81 to maximum 0.89 regarding STM, BL, and ESP ecotypes. However, with increasing Cu concentrations from 100 to 400 mg kg^{-1} no big differences were determined for RR under investigated giant reed ecotypes. Basically, adding Cu to soil-like growth medium enhanced the protein content of root system of all three studied ecotypes of giant reed, where plants grown on increasing Cu concentrations had higher protein contents of root system compared to control plants. Generally, protein content of plant culm of STM, BL, and ESP ecotypes growing on increasing Cu concentrations was similar to protein content of root system. The highest protein content in different plant parts of studied ecotypes was found in leaf blade. Moreover, STM, BL, and ESP ecotypes had the highest protein contents in their tissues of leaf blade at all treatments compared to protein contents in root systems and culm. Malondialdehyde (MDA) content of root, culm and leaf of BL, STM and ESP plants has been induced by adding Cu to soil-like growth medium. All ecotypes showed higher contents of MDA under different Cu treatments compared to control plant. This result may indicate the fatal effects of elevated Cu concentrations on plant cell wall composition and decomposing of phospholipid layer. BL, STM and ESP plants showed increasing contents of chl *a*, *b* and carotenoids.The highest content of chl *a* in STM and BL plants was measured at 400 mg Cu kg^{-1}, whilst ESP plants at 300 mg Cu kg^{-1} recorded the highest content of chl *a*. Cu treatments did not show any effects on Fv/Fm ratio for all ecotypes, where no significant differences were found between treatments of Cu and control plants. BL, STM and ESP plants had lower CO_2 assimilation values after 2 months (September) from starting the experiment, but 1 month later (October) the CO_2 assimilation values increased for all ecotypes compared to control. However, the highest Cu treatment significantly decreased CO_2 assimilation compared to other Cu treatments.

9. ÖSSZEFOGLALÁS

Két szomatikus embrió eredetű olasznád (*Arundo donax*) ökotípus — BL (amerikai ökotípus) és 20SZ (magyar ökotípus) — fitoremediációs potenciálját vizsgáltuk rézzel (Cu) mesterségesen szennyezett *in vitro* folyékony kultúrában. A két ökotípust steril körülmények között, tápoldatot tartalmazó csövekben, 6 hétig neveltük. A tápoldat növekvő dózisú Cu-et (0, 1, 2, 3, 5, 10 és 26,8 mg Cu L^{-1}) tartalmazott. A transzport és a bioakkumulációs faktorokat, valamint az eltávolítási hatékonyságot számoltuk. Egyik ökotípus esetében sem tapasztaltunk toxikus tüneteket az emelkedő Cu kezelések hatására 10 mg L^{-1} koncentrációig; azonban a BL ökotípusnál a 26,8 mg L^{-1} Cu kezelésnél rövid gyökér és szár hosszat, illetve sárgás levél elszíneződést figyeltünk meg. A növények a rezet nagyobbrészt gyökérszövetekben akkumulálták, a hajtás irányába kisebb mennyiségű réz szállítódott. Ezzel magyarázható, hogy toxikus tüneteket elsősorban a gyökérnövekedésben és a gyökér morfológiájában tapasztaltunk, t a hajtásrészeken kevésbé jelentek meg toxikus ünetek. A BL és a 20SZ ökotípus esetén a teljes felvett Cu több mint 59 és 58% halmozódott fel a gyökerekben. Ami a Cu-eltávolítási hatékonyságot illeti mindkét ökotípus magas értéket mutatott folyékony kultúrában: BL ökotípus esetében 96,6-98,8% között mozgott, 20SZ ökotípus esetén 97-100% között. Ezek az eredmények arra mutatnak, hogy mindkét olasznád ökotípus, alkalmas lehet Cu-el szennyezett vizes terület kezelésre, legfeljebb 26,8 mg L^{-1} koncentrációig.

Tudomásunk szerint jelenlegi, szomatikus embrió eredetű olasznád ökotípusokkal végzett kutatás az első olyan beszámoló, amely rámutatott a két ökotípusban rejlő fitoremediációs lehetőségre Cu-szennyezett vizek esetében.

A második kísérletben szilárd, *in vitro* kultúrában modellezztük a rézszennyezett talajok alkalmazhatóságát olasznád ökotípusok biomassszatermelésére, s ezzel együtt növények kármentesítési célra történő hasznosítását is vizsgáltuk.

A kísérletet BL és 20SZ ökotípusokkal végeztük. A táptalaj növekvő koncentrációban tartalmazott réz-szulfátot az alábbi sorrendben: 1, 2, 3, 5, 10, és 26,8 mg Cu L^{-1}. Egyik ökotípus sem mutatott észrevehető, Cu toxicitást jelző tüneteket a lombozaton a vizsgált koncentrációk hatására, 10 mg L^{-1}koncentrációig. Mindkét ökotípus esetén szignifikánsan növekedett a növények száraz tömege a legmagasabb Cu kezelés hatására a kontrollhoz képest. A BL ökotípus nagyobb Cu felvételi kapacitással rendelkezett, mint a 20SZ, mivel a száraz tömeg, valamint a hajtás hossz nagyobb volt a BL ökotípusnál a 20SZ-hez képest. A bio-koncentráció és a transzport faktorok értékei nagyobbak voltak a BL-nél, a 20SZ ökotípushoz képest. Közel 45% volt az összes Cu-tartalom a gyökérben, mindkét ökotípus esetén. Összességében eredményeinkből arra a következtetésre jutottunk, hogy olasznád alkalmas lehet Cu-szennyezett talajokon is biomasszatermelésre miközben segít a talaj kármentesítésében is.

A két ökotípust egymással összevetve azt találtuk, hogy a BL ökotípus különböző növényi szervei több Cu-t akkumuláltak, mint a 20SZ ökotípus, a BL ökotípus növényi részeinek száraz tömege nagyobb volt, mint 20SZ ökotípusnál, különösen a legmagasabb Cu koncentráció esetén. A gyökér, a hajtás és a levél száraz tömege 0,16; 0,14 és 0,31 g növény^{-1} volt a BL ökotípusnál és 0,15; 0,06 és 0,09 g növény^{-1} a 20SZ ökotípus esetén, illetve a 26,8 mg L^{-1} kezelésnél. Ezek az eredmények arra utalhatnak, hogy a BL ökotípus képes könnyebben és gyorsabban alkalmazkodni a magas Cu koncentrációhoz, mint a 20SZ a növényfejlődés e szakaszában. TLehetséges, hogy a BL ökotípusnak több Cu-ra van szüksége a növekedéshez, mint a 20SZ ökotípusnak, mivel a Cu egy esszenciális mikroelem. Mindazonáltal a BL ökotípus e viselkedése a Cu-el kapcsolatban nem egészen ismeretes, hiszen ahol a Cu magas koncentrációban van jelen ott Cu toxicitási tünetek tapasztalhatók. A 10 és 26,8 mg L^{-1} koncentrációjú Cu kezeléseknél a BL növények hajtásának hossza 9,0 cm volt, addig a 20SZ növényeknél ugyanilyen koncentrációjú kezeléseknél 14,5 és 6,5 cm volt egyenként. A 20SZ növények alacsonyabb koncentrációban akkumulálták a Cu-t a legnagyobb Cu kezelés esetén, azonban e növények hajtásának hosszára negatívan hatottak a növekvő Cu kezelések, a BL növényekkel összehasonlítva, amelyeknél a hajtások hosszabbak voltak ugyanazon kezelések esetén. Másrészt a BL növények gyökérzetét jelentősen befolyásolta a réz jelenléte.

A legmagasabb Cu koncentrációnál a BL gyökerek hossza rövidebb (2,5 cm) volt a 20SZ növények gyökereihez képest. Ennek magyarázata a gyökér Cu-tartalmában keresendő mindkét ökotípus esetén, mivel a BL gyökerekben a Cu tartalom majdnem kétszerese volt, mint a 20SZ gyökerekben. A BL növények száraz tömege nagyobb volt, mint a 20SZ növények száraz tömege, habár nagyobb Cu-tartalmat mértünk a BL esetén a 20SZ-hez képest. Mindkét ökotípus ugyanolyan Cu-felvételi tendenciát mutatott az alacsony Cu kezeléseknél, 5 mg L^{-1} koncentrációig. Alapvetően a tápközeg növekvő Cu-koncentrációjával a különböző növényi részekben is emelkedett a Cu-tartalom. A magasabb Cu koncentrációknál a BL ökotípus képes volt szignifikánsan több Cu felvételére a gyökér-, a hajtás- és a levélszövetekben a 20SZ ökotípushoz képest. A BL ökotípusnál a különböző növényi részek Cu tartalma a következő sorrendben alakult: gyökér > levél > hajtás, a 20SZ ökotípus esetén pedig a következő a sorrend: gyökér > hajtás > levél. Számszerűleg a BL növényeknél az összes Cu 41,4% volt a gyökérben és 23,3% a levélben, míg a szárban 21,0% Cu akkumulálódott. Másfelől a 20SZ növények gyökerében az összes Cu-tartalom 45,0% volt, a levélben 21,7% és 18,9% a növény hajtásában. Ezek az eredmények mutatják, hogy rézzel mérsékelten szennyezett területek alkalmasak lehetnek biomassza-termelési célokra, s mivel a réz nagyobb része az olasznád gyökerében halmozódik fel biztonságosabb a földfeletti részek betakarítása. A fitoextrakció kritériuma, hogy adott növény nagyobb mértékben tolerálja a szennyező anyagot, mint általában a növényfajok. Ezzel együtt lényeges szempont, hogy a felvett szennyező anyag nagyobb mértékben a föld feletti növényi részekben (hajtás és levelek) halmozódjon fel. A kísérletek alapján úgy tűnik, hogy a BL ökotípus jól tolerálja a rezet ,így alkalmas lehet biomassza termelésre szennyezett talajokon. Figyelembe véve a kiszámolt bioakkumulációs faktor értékeket a BL olasznád nem tekinthető ugyan réz-hiperakkumuláló növénynek.. de a hektáronkénti magas biomassza hozama és évelő életformája miatt jelentős mennyiségű Cu eltávolítását eredményezheti. Tekintve, hogy éves hozama 40 száraz tonna lehet hektáronként, BL ökotípusú olasznád betakarításával 40,73 mg kg^{-1} Cu távolítható el.

Harmadik kísérletünket üvegházi körülmények között tenyészedényben állítottuk be – 2013 júliusától novemberig – edényenként 2 palánta olasznáddal. A kísérlet során az alábbi ökotípusokat használtuk: STM (magyar), BL (amerikai) és ESP (spanyol). A vizsgálat célja az volt, hogy tanulmányozzuk a Cu hatását a növények túlélésére. Számos túlélő anyanövényt felvételeztünk a kísérleti időszak végén. Minden ökotípus jelentős toleranciát mutatott a magas Cu-koncentrációval szemben, ahol a magas Cu szintek nem befolyásolták negatívan az anyanövények számát akkor, amikor a kísérlet elkezdődött. Sőt, a növényenként megjelenő új hajtások számát pozitívan befolyásolták a növekvő Cu adagok, különösen az STM és a BL ökotípusok esetén. Növényenként 6 új hajtást mértünk a 200 és 300 mg kg^{-1} kezelés hatására az STM és a BL ökotípusoknál. Ezzel szemben 4,8 új hajtás fejlődött ESP ökotípusnál, amely nem kapott Cu ellátást. Az új tőhajtások hossza szignifikánsan növekedett a növekvő Cu kezelések hatására minden ökotípusnál a kontrollhoz képest. Az anyanövények és az új hajtások levelinek száma a réz kezelések függvényében hasonló tendenciát mutatott az egyes ökotípusoknál. A magas, 400 mg kg^{-1} Cu koncentráció jelentősen csökkentette az anyanövények internódiumainak hosszát az STM és a BL ökotípusoknál a kontroll növényhez képest. Az ESP ökotípust kevésbé befolyásolta negatívan a magas Cu-kezelés, mivel ez esetben a leghosszabb internódium a 200 mg Cu kg^{-1} kezelésnél volt megfigyelhető, a kontroll kezeléssel összehasonlítva. A kísérlet felszámolásakor a hajtás hossz-adatok arra engedtek következtetni, hogy az STM ökotípushoz tartozó növények jobban tolerálták a tápközeg növekvő Cu -tartalmát, mint a BL és ESP ökotípusok. A három ökotípust összehasonlítva az ESP bizonyult a legérzékenyebbnek a talaj emelkedő réztartalmára. A 100 mg kg^{-1} feletti rézkoncentráció szignifikánsan csökkentette az ESP növények hajtás növekedését a kontrollhoz képest.

A leveles hajtástól eltérően, a növények gyökérzetén megjelenő, magas Cu koncentrációk okozta káros tünetek mindhárom ökotípus esetében egyaránt megfigyelhetők voltak. Általánosságban elmondható, hogy a növekvő Cu szint szignifikánsan csökkentette a gyökerek hossznövekedését minden ökotípusnál, kivéve az STM gyökérzetet, a 100 mg Cu kg^{-1} kezelés esetén. Az STM növények hajtás térfogatának eredményei korreláltak a hajtáshossz eredményekkel. Minden egyes Cu kezelés pozitívan befolyásolta az STM növények növekedését, ezzel együtt a hajtás térfogat értékei is hasonló tendenciát mutattak.

A gyökérzetet vizsgálva azt találtuk, hogy gyökér térfogat a talaj réztartalmának növekedése mellett fokozatosan csökkent BL ökotípus esetében. STM és az ESP ökotípus esetében azonban nem találtunk ilyen szoros negatív összefüggést a talaj növekvő Cu szintje és a gyökér térfogat tekintetében. A friss tömeget vizsgálva csökkenést tapasztaltunk a növekvő Cu szintek hatására mindhárom ökotípus egyedeinél. A gyökérrel szemben sem a növények szárának sem levelének friss tömegét nem befolyásolta negatívan a talaj réztartalma 100-400 mg kg^{-1} koncentrációtartományban a három vizsgált ökotípus esetében. Általánosságban elmondható, hogy a kísérlet során a talajhoz adott növekvő Cu szinteknek nem volt egyértelmű hatása a három ökotípus gyökereinek száraz tömegére. Habár a BL növények negatív választ adtak a növekvő Cu dózisokra, a Cu szint emelésével, 300 mg kg^{-1} Cu-ig fokozatosan csökkent a gyökérzet száraz tömege, de ismét növekedés volt tapasztalható a 400 mg Cu kg^{-1} kezelésnél. A vizsgált óriás olasznád ökotípusok gyökérzetének száraz tömegével szemben a hajtás száraz tömegét pozitívan befolyásolták a növekvő Cu adagok. Minden ökotípusnál a hajtás száraz tömegének értéke nagyobb volt, mint a kontroll növények esetén. Az olasznád ökotípusok között az STM növényeknél volt a legalacsonyabb a levéllemez száraz tömege, illetve a 200 mg kg^{-1} feletti Cu kezeléseknél volt a legmagasabb a száraz tömeg, a BL és az ESP ökotípushoz képest.

A vizsgált három ökotípus (STM, BL és ESP) anyanövényeinek Cu-tartalma szignifikáns különbségeket mutatott a növekvő dózisú Cu-kezelések hatására. Az STM ökotípus anyanövényeinek hajtásrészében mértük a legnagyobb Cu-tartalmat a 100 és 300 mg Cu kg^{-1} kezelések hatására, a másik két ökotípushoz képest. Azonban az összes ökotípusnál, az anyanövények hajtás részében volt a legmagasabb, 121 µg g^{-1} a Cu tartalom, illetve az STM növényekben a 300 mg kg^{-1} kezelésnél. A vizsgált STM, BL és ESP olasznád ökotípusok gyökérzetének Cu tartalma szignifikánsan növekedett a kísérleti tenyészedényekben lévő emelkedő Cu koncentrációk hatására. Azonban a növekvő Cu szintek hatására minden ökotípus növekvő mennyiségű Cu-t akkumulált a gyökérzetében; a 400 mg Cu kg^{-1} kezelésnél a Cu tartalom a gyökérzetben 3-szor több volt, mint a 100 mg Cu kg^{-1}–val kezelt ökotípus gyökérzetében. Az ESP növény hajtásának Cu tartalma egyenesen arányos volt a táptalajban alkalmazott Cu dózisokkal. Ahogy a kezelésekben növekedett a Cu koncentrációja, úgy koncentrálódott a Cu az ESP növények hajtásának szöveteiben 43-101 µg g^{-1} tartományban, a 100-400 mg Cu kg^{-1} kezelések esetén. Az ökotípusok között a legmagasabb Cu-tartalmat (130 µg g^{-1}) a BL ökotípus hajtásában, a 200 mg kg^{-1} kezelésnél mértük.

Alapvetően a növekvő Cu szintek nem növelték lényegesen a levelek összes Cu-tartalmát a vizsgált ökotípusok esetén, kivéve az ESP növényt, ahol a legmagasabb mért Cu-tartalmat (84 µg g^{-1}) a legnagyobb Cu kezelésnél, 400 mg kg^{-1}-nál figyeltük meg. A növények betakarítását követően a talajmintákban visszamaradt valamennyi hozzáférhető réz, melynek koncentrációja arányosan növekedett az alkalmazott kezelésekkel.

Alapvetően megnövekedett felvehető Cu koncentrációkat mértünk a tenyészedényekben az STM, a BL és az ESP ökotípus kísérlet befejezését követően. Lényegében a tápközeghez adott növekvő Cu dózisok az összes Cu koncentrációjának szignifikáns növekedését eredményezték minden tenyészedényben, a vizsgált összes ökotípus esetén. Az olasznád ökotípusok összes Cu koncentrációra gyakorolt hatása egyértelműen megfigyelhető volt. Nem találtunk szignifikáns különbségeket az összes

Cu koncentráció esetén a 100 és 200 mg Cu kg^{-1} kezeléseknél, azonban a növekvő Cu hatására exponenciális növekedést figyeltünk meg a kezelésekben. A transzport faktor (TF) számított értékeit szignifikánsan befolyásolta a táptalaj növekvő Cu koncentrációja. Az STM, a BL és az ESP ökotípus esetén egyaránt a TF értékek kisebbek voltak mint egy, ami utalás arra, hogy az olasznád kis mértékben szállítja a rezet a gyökérzetből (földalatti rész) a hajtásrendszerbe (föld feletti rész). Ez rámutat arra, hogy teljes és hatékony fitoremediációs folyamatra van szükség, tehát a teljes növényt el kell távolítani, beleértve a gyökérzetet is, melynek eltávolítása fontos. A becsült TF-értékek először növekedtek, majd szignifikánsan csökkentek a növekvő, 200 mg kg^{-1} feletti Cu koncentrációk hatására, az összes vizsgált ökotípus esetén. A TF értékekkel ellentétben a biokoncentrációs faktor (BCF - bioconcentration factor) >1 értékeket mutattak mindhárom vizsgált ökotípus esetében 100-400 mg Cu kg^{-1} kezelési tartományban. A növekvő Cu koncentráció szignifikáns különbségeket eredményezett a BCF értékekben a vizsgált ökotípusok között. Lényegében minden vizsgált ökotípus azonos hatékonysággal jellemezhető a Cu talajból történő eltávolítását illetően. Az eltávolítási hatékonyság (RR - removal rate) értékek minimum 0,81 és maximum 0,89 között változtak. Azonban a növekvő 100 - 400 mg kg^{-1} közötti Cu koncentrációk hatására az RR értékek esetén nem tapasztaltunk nagy különbségeket a vizsgált olasznád ökotípusok között.

A táptalajhoz adott növekvő Cu dózisok növelték a gyökérzet fehérjetartalmát, mindhárom vizsgált olasznád ökotípus esetén a kontrollhoz viszonyítva. Általában a növekvő Cu koncentrációjú tápközegben nevelt STM, BL és ESP ökotípusok hajtásának fehérjetartalma hasonló volt a gyökérzet fehérjetartalmához. A vizsgált ökotípusok különböző növényi részei közül a levéllemez fehérjetartalma volt a legnagyobb. Az STM, a BL és az ESP ökotípusok fehérjetartalma a levéllemez szöveteiben volt a legmagasabb minden kezelés, illetve a gyökérzet és a hajtás fehérjetartalmának összehasonlításában. A gyökér, hajtás és levél malondialdehid tartalma növekedett a rézkezelések hatására a kontrollhoz viszonyítva mindhárom ökotípus esetében. Ezek az eredmények arra utalnak, hogy a növekvő réz koncentráció károsítja a sejtek lipidmembrán szerkezetét. Ugyanakkor növekvő tendenciát figyeltünk meg a fotoszintetikus pigmenttartalomban (chl *a*, *b* és karotinoidok) is BL, STM és ESP ökotípusok esetében egyaránt. A legmagasabb chl a értékeket STM és BL ökotípusoknál 400 mg Cu kg-1kezeléseknél találtunk, míg ESP ökotípus esetében 300 mg Cu kg^{-1} –nál. Az Fv/Fm arányban, mely a növények aktuális fotokémiai hatékonyságára utal, nem találtunk szignifikáns különbséget a kontrollt és az egyes kezeléseket vizsgálva. Ezzel szemben a CO_2 beépülés mértékében jelentős különbségeket tapasztaltunk az ökotípusokat összehasonlítva, figyelembe véve a kezeléseket. A növekvő Cu kezeléssel együtt kifejezett növekedést tapasztaltunk a szén-dioxid asszimiláció mértékében BL és STM ökotípusok esetében. A legmagasabb értéket 300 mg Cu kg^{-1} kezelésnél találtuk.

10. REFERENCES

Adler P. R. – del-Grosso S. J. – Parton W. J. (2007): Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. Ecological Applications. **17**. 3. 675–691.

Adriano D. C. (2001): Trace elements in the terrestrial environments: biogeochemistry, bioavailability, and risks of metals. New York–Berlin–Heidelberg–Tokyo: Springer – Verlag.

Allen H. E. (1993): The significance of trace metal speciation for water, sediment and soil quality criteria and standards. Sci. Total Environ. Suppl.1:23–45.Alloway B. J. (1995). Heavy metals in soils. 2nd ed., Chapman & Hall, London.

Alloway B. J. (1995): Heavy metals in soils. 2nd ed., Chapman & Hall, London.

Alshaal T. – Domokos-Szabolcsy É. – Márton L. – Czakó M. – Kátai J. – Balogh P. – Elhawat N. – El-Ramady H. – Fári M. (2013): Phytoremediation of bauxite-derived red mud by giant reed (*Arundo donax* L.). Environmental Chemistry Letters. **11**. 3. 295-302. doi: 10.1007/s10311-013-0406-6

Alshaal T. – Domokos-Szabolcsy É. – Márton L. – Czakó M. – Kátai J. – Balogh P. – Elhawat N. – El-Ramady H. – Gerőcs A. – Fári M. (2014): Restoring soil ecosystems and biomass production of *Arundo donax* L. under microbial communities-depleted soil. Bioenergy Research. **7**. 1. 268-278. doi: 10.1007/s12155-013-9369-5

Alshaal T. – Elhawat N. – Domokos-Szabolcsy É. – Kátai J. – Márton L. – Czakó M. – El-Ramady H. – Fári M. (2015): Giant Reed (*Arundo donax* L.): A Green Technology for A Clean Environment. In: *Ansari A. A.* (eds) Phytoremediation: Management of Environmental Contaminants. Vol. 1. Springer Science + Business Media, LLC, XIV. ISBN: 978-3-319-10394-5. pp. 3-20. doi: 10.1007/978-3-319-10395-2_1

Aoyama M. – Nagumo T. (1997): Comparison of the effects of Cu, Pb, and As on plant residue decomposition, microbial biomass and soil respiration. Soil Science and Plant Nutrition. **43**. 613-622.

Arun K. S. – Carlos C. – Herminia L. Z. – Avudainayagam S. (2005): Chromium toxicity in plants. Environmental International. **31**. 739-753.

Ashraf M. – Harris P. J. C. (2013): Photosynthesis under stressful environments: An overview. Photosynthetica. **51**. 2. 163-190 doi: 10.1007/s11099-013-0021-6

Bacher W. – Mix-Wagner G. – Sauerbeck G. – El Bassam N. (2001): Giant Reed (*Arundo donax* L.) Network - Improvement Biomass Quality, Final Report, FAIRCT-96-2028, Participant 9, FAL (Institute of Crop Science), Braunschweig, 1-72.

Baker A. J. – Brooks R. R. (1989): Terrestrial higher plants which hyperaccumulate metallic elements: a review of their distribution, ecology and phytochemistry. Biorecovery. **1**. 81–126.

Baker D. E. – Senef J. P. (1995): Copper. In: Alloway BJ (ed) Heavy Metals in Soils, pp. 179-205. Blackie Academic and Professional, London.

Bañuelos G. S. (2006): Phyto-products may be essential for sustainability and implementation of phytoremediation. Environmental Pollution. **144**. 19–23.

Barman S. C. – Sahu R. K. – Bhargava S. K. – Chatterjee C. (2000): Distribution of heavy metals in wheat, mustard, and weed grown in fields irrigated with industrial effluents. Bulletin of Environmental Contamination and Toxicology. **64**. 489–496. doi:10.1007/s001280000030

Baroux J. (1972): Toxicity of copper on ammonification phenomena in soils of Bordeaux vineyards. C R Hebd Seances Acad Sci Ser D. **275**. 499–502.

Bell G. (1997): Ecology and management of Arundo donax , and approaches to riparian habitat restoration in Southern California. In: *Brock J. H. – Wade M. – Pysek P. – Green D.* (eds) Plant invasions: Studies from North America and Europe. Blackhuys, Leiden, pp. 103–113.

Beringer T. I. M. – Lucht W. Schaphoff S. (2011): Bioenergy production potential of global biomass plantations under environmental and agricultural constraints. GCB Bioenergy. **3**. 299–312

Bernal M. – Roncel M. – Ortega J. M. – Picorel R. – Yruela I. (2004): Copper effect on cytochrome b559 of photosystem II under photoinhibitory conditions. Physiology Plant. **120**. 686-694. doi : 10.1111/j.1399-3054.2004.0286.x

Blake A. – Clarke M. – Stuart-Street A. (2012): Changing land use on unproductive soils. *Resource management technical report 379*, Department of Agriculture and Food Western Australia.

Bolan N. D. – Adriano S. M. – Khan A. (2003): Adsorption, complexation and phytoavailability of copper as influenced by organic manure. Environmental Toxicology and Chemistry **22**. 450–456.

Bonanno G. (2012): *Arundo donax* as a potential biomonitor of trace element contamination in water and sediment. Ecotoxicology and Environmental Safety. **80**. 20–27. doi: 10.1016/j.ecoenv.2012.02.005

Bouazizi H. – Jouili H. – Geitmann A. – Ferjani E. E. I. (2010): Copper toxicity in expanding leaves of *Phaseolus vulgaris* L.: antioxidant enzyme response and nutrient element uptake. Ecotoxicology and Environmental Safety. **73**. 6. 1304–1308.

Boucher P. (2012): The role of controversy, regulation and engineering in the UK biofuel development. Energy Policy **42**. 148–154. http://dx.doi.org/10.1016/j.enpol.2011.11.058.

BP, (2012): Statistical Review of World Energy. Published by: Addy Rikka on Feb 04, 2013.

Bradford M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. **72**. 248-254

Bradl H. B. (2004): Adsorption of heavy metal ions on soils and soils constituents. Journal of Colloid and Interface Science. **277**. 1–18.

Brian K. R. – Cathelijne R. S. – Ian J. C. – Peter B. W. (2014): Reporting on Marginal Lands for Bioenergy Feedstock Production: a Modest Proposal. Bioenergy Research. **7**. 1060–1062. doi 10.1007/s12155-014-9408-x

Broadley M. – Brown P. – Cakmak I. – Rengel Z. – Zhao F. (2012): Function of Nutrients: Micronutrients. In: P. Marschner (ed.) Marschner's Mineral Nutrition of Higher Plants. pp. 191 – 248. doi:10.1016/B978-0-12-384905-2.00007-8

Brookes P. C. (1995): The use of microbial parameters in monitoring soil pollution by heavy metals. Biology and Fertility of Soils. **19**. 269–279.

Brooks R. R. (1977): Copper and cobalt uptake by *Haumaniastrum* species. Plant and Soil. **48**. 541-544.

Cai X. – Zhang X. – Wang D. (2011): Land availability for biofuel production. Environmental Science & Technology. **45**. 334-339.

Calabrese E. J. (2004): Hormesis: from marginalization to mainstream: a case for hormesis as the default dose-response model in risk assessment. Toxicology and Applied Pharmacology. **197**. 125–136.

Campbell J. E. – Lobell D. B. – Genova R. C. – Field C. B. (2008): The global potential of bioenergy on abandoned agriculture lands. Environmental Science & Technology. **42**. 5791–5794. doi:10.1021/es800052w

Chamberlain A. C. (1983): Fallout of lead and uptake by crops. Journal of Atmospheric Environment. **17**. 693–706.

Chaney R. L. (1983): Plant uptake of inorganic waste. In: *Parr J. E. et al.* (eds) Treatment of hazardous wastes. Noyes Data, Park Ridge, IL, pp. 50–76.

Chaoui A. – Mazhoudi S. – Ghorbal M. H. – Ferjani E. (1997): Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). Plant Science. **127**. 139–147.

Chen C. T. –Chen L. M. –Lin C. C. –Kao C. H. (2001): Regulation of proline accumulation in detached rice leaves exposed to excess copper. Plant Science. **160**. 283-290.

Chopin E. I. B. – Marin B. – Mkoungafoko R. – Rigaux A. – Hopgood M. J. – Delannoy E. – Cancès B. – Laurain M. (2008): Factors affecting distribution and mobility of trace elements (Cu, Pb, Zn) in a perennial grapevine (*Vitis vinifera* L.) in the Champagne region of France. Environmental Pollution. **156**. 3. 1092–1098.

Clemens S. (2001): Molecular mechanisms of plant metal tolerance and homeostasis. Planta. **212**. 475–486.

Coffman G. C. – Ambrose R. F. – Runde P. W. (2010): Wildfire promotes dominance of invasive giant reed (*Arundo donax*) in riparian ecosystems. Biological Invasions. **12**. 8. 2723–2730. doi: 10.1007/s10530-009-9677-z

Cornforth I. S. – Bussell W. T. – Lewthwaite J. R. (2003): Significance of soil copper in the urban development of horticultural soils. New Zealand Journal of Crop and Horticultural Science. **31**. 4. 293-302.

Crutzen P. J. – Mosier A. R. – Smith K. A. – Winiwarter W. (2008): N_2O release from agrobiofuel production negates global warming reduction by replacing fossil fuels. Atmospheric Chemistry and Physics. **8**. 389–395.

Dafang Z. – Dong J. – Lei L. – Yaohuan H. (2011): Assessment of bioenergy potential on marginal land in China. Renewable and Sustainable Energy Reviews. **15**. 1050–1056. doi:10.1016/j.rser.2010.11.041

Dey S. – Mazumder P. B. – Paul S. B. (2014): Effect of copper on growth and chlorophyll content in tea plants (*Camellia sinensis* (L.) O. kuntze). International Journal of Research in Applied, Natural and Social Sciences. **2**. 5. 223-230.

Díaz-Raviña M. – Calvo de Anta R. – Bååth E. (2007): Tolerance (PICT) of the bacterial communities to copper in vineyards soils from Spain. Journal of Environmental Quality. **36**. 1760–1764.

Dietz K. J. – Bair M. – Krämer U. (1999): Free radicals and reactive oxygen species as mediators of heavy metals toxicity in plants. In: *Prasad M. N. V.* and *Hagemeyer J.* (eds), Heavy Metal Stress in Plants from Molecules to Ecosystems, Springer-Verlag, Berlin. pp. 73–97.

Ding H. D. – Wan Y. H. – Qi N. M. – Zhu W. M. – Yng X. F. – Shao Y. C. (2004): Effects of Cd^{2+} and Zn^{2+} stress on antioxidant enzyme system of tomato seedlings. Acta Agriculturae Shanghai. **20**. 79–82.

Donner S. D. – Kucharik C. J. (2008): Corn-based ethanol production compromises goal of reducing nitrogen export by the Mississippi River. Proceedings of the National Academy of Science of the USA. **105**. 4513–4518.

Dykeman W. R. – De Sousa A. S. (1966): Natural mechanisms of copper tolerance in a copper swamp. Canadian Journal of Botany. **44**. 7. 871-878.

EC, European Commission (2002): EC (European Commission) regulation 473/2002 amending Annexes I, II and VI to Council Regulation (EEC) No 2092/91 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs, and laying down detailed rules as regards the transmission of information on the use of copper compounds.

EC, European Commission. Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the promotion of the use of energy from renewable sources. COM (2008) 19 final. Brussels: Commission of the European Communities; 23.01.2008.

Elhawat N. – Alshaal T. – Domokos-Szabolcsy É. – El-Ramady H. – Márton L. – Czakó M. – Kátai J. – Balogh P. – Sztrik A. – Molnár M. – Popp J. – Fári M. (2014): Phytoaccumulation potentials of two biotechnologically propagated ecotypes of *Arundo donax* in copper-contaminated synthetic wastewater. Environmental Science and Pollution Research. **21**. 12. 7773-7780. doi: 10.1007/s11356-014-2736-8

Elhawat N. – Alshaal T. – Kratz S. – Domokos-Szabolcsy É. – El-Ramady H. – Prokisch J. – Eszenyi P. – Sztrik A. – Babka B. – Fári M. (2013): Ecotoxicology of copper in horticultural soils: A review. International Journal of Horticultural Sciences. **19**. 1-2. 7-18.

ExxonMobil. (2013): The Outlook for Energy: A View to 2040, Corporate Headquarters, 5959 Las Colinas Blvd. Irving, Texas 75039-2298.

FAO (2011): Energy-smart food for people and climate. Issue paper. Rome: FAO; 2011

FAO (1997) : Report of the study on CGIAR research priorities for marginal lands. Consultative Group on International Agricultural Research, Food and Agriculture Organization of the United Nations; Available at: http://www.fao.org/Wairdocs/TAC/X5784E/x5784e02.htm. Accessed 18 Jun 14

Fargione J. – Hill J. – Tilman D. – Polasky S. – Hawthorne P. (2008): Land clearing and the biofuel carbon debt. Science. **319**. 1235–1238.

Fernández-Calviño D. – Arias-Estévez M. – Díaz-Raviña M. – Bååth E. (2011a): Bacterial pollution induced community tolerance (PICT) to Cu and interactions with pH in long-term polluted vineyard soils. Soil Biology and Biochemistry. **43**. 2324–2331.

Fernández-Calviño D. – Rousk J. – Brookes P. C. – Bååth E. (2011b): Bacterial pH-optima for growth track soil pH, but are higher than expected at low pH. Soil Biology Biochemistry. **43**. 1569–1575.

Fernández-Calviño D. – Soler-Rovira P. – Polo A. – Díaz-Raviña M. – Arias-Estévez M. – Plaza C. (2010a): Enzyme activities in vineyard soils long-term treated with copper-based fungicides. Soil Biology and Biochemistry. **42**. 2119–2127.

Fernández-Calviño D. – Martín A. – Arias-Estévez M. – Bååth E. – Díaz-Raviña M. (2010b): Microbial community structure of vineyard soils with different pH and copper content. Applied Soil Ecology. **46**. 276–282.

Fernández-Calviño D. – Pérez-Novo C. – Nóvoa-Muñoz J. C. – Arias-Estévez M. (2009a): Copper accumulation and fractionation in vineyard soils from temperate humid zone (NW Iberian Peninsula). Geoderma. **153**. 119-129.

Fernández-Calviño D. – Pérez-Novo C. – Nóvoa-Muñoz J. C. – Arias-Estévez M. (2009b): Copper fractionation and release from soils devoted to different crops. Journal of Hazardous Materials. **167**. 797-802.

Fischer G. – Prieler S. – van Velthuizen H. T. (2010): Biofuel production potentials in Europe: sustainable use of cultivated land and pastures. Part I: Land productivitry potentials. Biomass and Bioenergy. 34. 2. 159-172. doi:10.1016/j.biombioe.2009.07.008.

Fischer-Boel M. (2008): Biofuels: more valuable as fuel than as a scapegoat. Policy dialogue on biofuels organised by EPC (European Policy Centre). Brussels; 6 May 2008. SPEECH/08/228.

Frandsen P. R. (1997): Team Arundo: interagency cooperation to control giant reed cane (*Arundo donax*). In: *James O.* (ed) Assessment and management of plant invasions. Springer, New York.

Gaw S. K. (2002): Pesticide residues in horticultural soils in the Auckland Region. Working Report No. 96. Auckland, Auckland Regional Council. 91 p.

Ge X. – Burner D. M. – Xu J. – Phillips G. C. – Sivakumar G. (2011): Bioethanol production from dedicated energy crops and residues in Arkansas, USA. Biotechnology Journal. **6**. 66–73.

Gelfand I. – Sahajpal R. – Zhang X. – Izaurralde R. C. – Gross K. L. – Robertson G. P. (2013): Sustainable bioenergy production from marginal lands in the US Midwest. Nature. **493**. 7433. 514–517.

Nagy G. – Kovács L. – Ünnep R. – Zsiros O. – Almásy L. – Rosta L. – Timmins P. – Peters J. – Posselt D. – Garab G. (2013): Kinetics of structural reorganizations in multilamellar photosynthetic membranes monitored by small-angle neutron scattering. The European Physical Journal E. **36**. 69-81. doi: 10.1140/epje/i2013-13069-0

Goldemberg J. (2007): Ethanol for a sustainable energy future. Science. **315**. 808–810. http://dx.doi.org/10.1126/science.1137013.

Günes K. – Saygin Ö. (1996): Productivity of the energy crops: giant reed and sweet sorghum in Turkey. Fresenius Environmental Bulletin. **5**. 11-12. 756–761.

Gunkel P. – Roth E. – Fabre B. (2003): Copper distribution in chemical soil fractions and relationships with maize crop yield. Environmental Chemistry Letter. **1**: 92–97.

Hall J. L. (2002): Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany. **53**. 1-11. doi: 10.1093/jexbot/53.366.1

Halliwell B. – Gutteridge J. M. C. (1984): Oxygen toxicity, oxygen radicals, transition metals and disease. Biochemistry Journal. **219**. 1-14.

Harrison R. – Chirgawi M. B. (1989): The assessment of air and soil as contributors of some trace metals to vegetable plants I. Use of a filtered air growth cabinet. The Science of the Total Environment. **83**. 13–34.

Hasselgren K. (1999): Utilization of sewage sludge in short-rotation energy forestry: a pilot study. Waste Management Research. **17**. 251–262.

Hatfield J. et al. (2014): Agriculture. In: Melillo J. M. – Richmond T. C. –Yohe G. W. (eds) Climate change impacts in the United States: the third national climate assessment. U.S. Global Change Research Program, Washington, D.C, pp. 150–174.

Hattori T. – Morita S. (2010): Energy crops for sustainable bioethanol production; which, where and how? Plant Production Science. **13**. 3. 221–234.

He Z. L. – Fan X. – Yang X. E. – Stoffella P. J (2010): Soil amendment to remediate copper contaminated soils. 19th World Congress of Soil Science, Soil Solutions for a Changing World 1 – 6 August 2010, Brisbane, Australia, pp. 227 – 230.

He Z. L. – Yang X. E. – Stoffella P. J. (2005): Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology. **19**. 125–140.

Hill J. – Nelson E. – Tilman D. – Polasky S. – Tiffany D. (2006): Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. Proceedings of the Academy of Sciences of the United States of America. **103**. 30. 11206–11210.

Hill J. – Polasky S. – Nelson E. – Tilman D. – Huo H. (2009): Climate change and health costs of air emissions from biofuels and gasoline. Proceedings of the Academy of Sciences of the United States of America. **106**. 2077–2082.

IEA, (2009): Bioenergy. A sustainable and reliable energy source. Main Report. International Energy Agency, Paris.

James, R. O. – Barrow N. J. (1981): Copper interactions with inorganic constituents of soils. In: *Loneragan J. F. – Robson A. D. – Graham R. D.* (eds) Copper in soils and plants. Sydney, Academic Press. pp. 287-312.

Jeon Y. J. – Xun Z. – Rogers P. L. (2010): Comparative evaluations of cellulosic raw materials for second generation bioethanol production. Letters in Applied Microbiology. **51**. 518-524.

Jingying F. – Dong J. – Yaohuan H. – Dafang Z. – Wei J. (2014) : Evaluating the Marginal Land Resources Suitable for Developing Bioenergy in Asia. Advances in Meteorology, Volume 2014, Article ID 238945, 9 pages, http://dx.doi.org/10.1155/2014/238945

Jones J. B. Jr. (2003): Agronomic handbook – Management of crops, soils, and their fertility. CRC Press, Boca Raton, FL.

Jones J. B. Jr. (2005): Hydroponics — a practical guide for the soilless grower, 2nd ed. CRC PRESS, Boca Raton London, New York, Washington, D.C

Kabata-Pendias A. – Mukherjee A. B. (2007): Trace Elements from Soil to Human. Springer Science + Business Media, Springer-Verlag Berlin Heidelberg.

Kabata-Pendias A. – Sadurski W. (2004): Trace elements and compounds in soil. In: *Merian E. – Anke M. – Ihnat M. – Stoeppler M.* (eds) Elements and Their Compounds in the Environment, Wiley-VCH, Weinheim. pp. 79–99.

Kabata-Pendias A. (2011): Trace Elements in Soils and Plants, 4th ed., CRC Press, Boca Raton, FL. Taylor and Francis Group, LLC.

Karlsson T. – Persson P. – Skyllberg U. (2006): Complexation of copper (II) in organic soils and in dissolved organic matter — EXAFS evidence for chelate ring structures. Environmental Sciences & Technology. **40**. 2623–2628.

Karp A. – Shield I. (2008): Bioenergy from plants and the sustainable yield challenge. New Phytologist. **179**. 1. 15–32.

Khatun S. – Ali M. B. – Hahn E. J. – Paek K. Y. (2008): Copper toxicity in *Withania somnifera*: growth and antioxidant enzymes. Environmental and Experimental Botany. **64**. 279–285.

Komárek M. – Balík J. – Chrastný V. – Száková J. (2009): Distribution and fractionation of copper in contaminated hop field soils. Fresenius Environmental Bulletin. **18**. 1319–23.

Komárek M. – Čadková E. – Chrastný V. – Bordas F. – Bollinger J. C. (2010): Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environment International. **36**. 138–151. doi:10.1016/j.envint.2009.10.005.

Kupper H. – Setlik I. – Setlikova E. – Ferimazova N. – Spiller M. – Kupper F. C. (2003): Copper induced inhibition of photosynthesis: Limiting steps of *in vivo* copper chlorophyll formation in *Scenedesmus quadricauda*. Functional Plant Biology. **30**. 1187-1196.

Landis, D.A., Gardiner, M.M., van der Werf, W., Swinton, S.M. (2008): Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. Proceedings of the National Academy of Sciences of United States of America. **105**. 20552–20557.

Lauren D. Q. – Kaitlin C. S. – Jia G. – Kim S. – Santanu T. – Gary K. – Lee D. K. – Thomas B. V. (2015) : Stress-Tolerant Feedstocks for Sustainable Bioenergy

Production on Marginal Land. Bioenergy Research. doi: 10.1007/s12155-014-9557-y

Lequeux H. – Hermans C. – Lutts S. – Verbruggen N. (2010): Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiology and Biochemistry. **48**. 8. 673–682.

Li W. – Zhang M. – Shu H. (2005): Distribution and fractionation of copper in soils of apple orchards. Environmental Science and Pollution Research. **12**. 3. 168–172.

Lin C. – Lin Y. – Liu J. (2007): A study on the diversity and exploiting prospect of energy plant resources. Journal of Henan Agricultural Sciences. **12**. 17–21.

Lindsay W. L. (1979): Chemical equilibria in soils. New York–Chichester–Brisbane–Toronto. John Wiley & Sons.

Liu W. – Li P. J. – Qi X. M. – Zhou Q. X. – Zheng L. – Sun T. H. – Yang Y. S. (2005): DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. Chemosphere. **61**. 158-167

Lobell D. B. – Schlenker W. – Costa-Roberts J. (2011): Climate trends and global crop production since 1980. Science. **333**. 6042. 616-620. doi: 10.1126/science.1204531

Logan E. M. – Pulford I. D. – Cook G. T. – Mackenzie A. B. (1997): Complexation of Cu^{2+} and Pb^{2+} by peat and humic acid. European Journal of Soil Science. **48**. 4. 685–696. doi: 10.1111/j.1365-2389.1997.tb00568.x

Loland J. Ø. – Singh B. R. (2004): Copper contamination of soil and vegetation in coffee orchards after long-term use of Cu fungicides. Nutrient Cycling in Agroecosystems. **69**. 3. 203–211. doi: 10.1023/B:FRES.0000035175.74199.9a

Lovett A. – Sünnenberg G. – Dockerty T. (2014): The availability of land for perennial energy crops in Great Britain. Global Change Biology Bioenergy. **6**. 2. 99–107. doi: 10.1111/gcbb.12147

Lu L. – Jiang D. – Zhuang D. – Huang Y. (2012): Evaluating the marginal land resources suitable for developing pistacia chinensis-based biodiesel in China. Energies. **5**. 7. 2165–2177. doi:10.3390/en5072165

Luna C. M. – Gonzalez V. S. – Trippi V. S. (1994): Oxidative damage caused by excess copper in oat leaves. Plant and Cell Physiology. **35**. 11–15.

Ma Y. B. – Lombi E. – Nolan A. L. – McLaughlin M. (2006): Short-term natural attenuation of copper in soils: effect of time, temperature, and soil characteristics. Environmental Toxicology and Chemistry. **25**. 3. 652–658. doi: 10.1897/04-601R.1

Macnair M. R. – Tilstone G. H. – Smith S. E. (2000): The genetics of metal tolerance and accumulation in higher plants. In: *Terry N. – Banuelos. G.* (eds), Phytoremediation of contaminated soil and water. CRC Press LLC. pp. 235-250.

Maksymiec W. – Krupa Z. (2007): Effects of methyl jasmonate and excess copper on root and leaf growth. Biologia Plantarum. **51**. 2. 322–326. doi: 10.1007/s10535-007-0062-4

Malik A. (2007): Environmental challenge vis a vis opportunity: the case of water hyacinth. Environment International. **33**. 1. 122–138. doi:10.1016/j.envint.2006.08.004

Maltby L. – Brock T. C. M. – van den Brink P. J. (2009): Fungicide risk assessment for aquatic ecosystems: importance of interspecific variation, toxic mode of action, and exposure regime. Environmental Science & Technology. **43**. 19. 7556–7563. doi: 10.1021/es901461c

Manivasagaperumal R. – Vijayarengan P. – Balamurugan S. – Thiyagarajan G. (2011): Effect of copper on growth, dry matter yield and nutrient content of *Vigna radiata* (L.) Wilczek. Journal of Phytology. **3**. 3. 53-62.

Marschner H. (1995): Mineral nutrition of higher plants. 2nd ed., Academic, London.

Martin N. A. (1986): Toxicity of pesticides to *Allobophera caliginosa* (Oligochaeta: Lumbricidar). New Zealand Journal of Agricultural Research. **29**. 699-706.

Martínez-Villegas N. – Martínez C. E. (2008): Solid- and solution-phase organics dictate copper distribution and speciation in multicomponent systems containing ferrihydrite, organic matter, and montmorillonite. Environmental Science & Technology. **42**. 8. 2833–2838. doi: 10.1021/es072012r

Márton L. – Czakó M. (2002): Szelektált egyszikü nemzetségek hosszan tartó totipotens tenyészete. [Magyar szabadalmi bejelentés P0500786]

Márton L. – Czakó M. (2004): Sustained Totipotent Culture of Selected Monocot Genera. USA, patent 6,821,782.

Márton L. – Czakó M. (2007): Sustained Totipotent Culture of Selected Monocot Genera. USA, patent 7,303,916.

Mattson M. P. (2008): Dietary factors, hormesis and health. Ageing Research Reviews **7**. 43–48.

Mavrogianopoulos G. – Vogli V. – Kyritsis S. (2002): Use of wastewater as a nutrient solution in a closed gravel hydroponic culture of giant reed (*Arundo donax*). Bioresource Technology. **82**. 103–107.

Méchin V. – Argillier O. – Menanteau V. – Barrière Y. – Mila I. – Pollet B. – Lapierre C. (2000): Relationship of cell wall composition to in vitro cell wall digestibility of maize inbred line stems. Journal of The Science of Food and Agriculture. **80**. 574-580.

Meng Q. – Zou J. – Zou J. – Jiang W. – Liu D. (2007): Effect of Cu^{2+} concentration on growth, antioxidant enzyme activity and malondialdehyde content in garlic (*Allium sativum* L.). Acta Biologica Cracoviensia Series Botanica. **49**. 1. 95-101.

Mengel, K. – Kirkby E. A. – Kosegarten H. – Appel T. (2001): Principles of plant Nutrition. 2nd ed., International Potash Inst., Berne, Switzerland.

Miao Y. – Xiao X. – Miao X. – Guo Z. – Wang F. (2012): Effect of amendments on growth and metal uptake of giant reed (*Arundo donax* L.) grown on soil contaminated by arsenic, cadmium and lead. Transactions of Nonferrous Metals Society of China. **22**. 6. 1462–1469. doi: 10.1016/S1003-6326(11)61342-3

Michaud A. M. – Bravin M. N. – Galleguillos M. – Hinsinger P. (2007): Copper uptake and phytotoxicity as assessed in situ for durum wheat (*Triticum turgidum* durum L.) cultivated in Cu contaminated, former vineyard soils. Plant and Soil. **298**. 1-2. 99-111. doi: 10.1007/s11104-007-9343-0

Milbrandt A. – Overend R. P. (2009): Assessment of biomass resources from marginal lands in APEC economies. Energy Working Group, NREL/TP-6A2-46209. http://www.nrel.gov/docs/fy10osti/46209. pdf. Accessed 18 Jun 14

Milenkovski S. – Baath E. – Lindgren P. E. – Berglund O. (2010): Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. Ecotoxicology. **19**. 285–294.

Mirza N. – Mahmood Q. – Pervez A. – Ahmad R. – Farooq R. – Shah M. M. – Azim M. R. (2010): Phytoremediation potential of *Arundo donax* in arsenic-contaminated synthetic wastewater. Bioresource Technology. **101**. 15. 5815-5819. doi: 10.1016/j.biortech.2010.03.012

Mirza N. – Pervez A. – Mahmoud Q. – Shah M. M. – Shafqat M. N. (2011): Ecological restoration of arsenic contaminated soil by *Arundo donax* L. Ecological Engineering. **37**. 12. 1949–1956. doi:10.1016/j.ecoleng.2011.07.006

Mochizuki M. M. – Tellis A. – Wills M. (2004): Confronting terrorism in the pursuit of power: Strategic Asia, 2004-2005. The National Bureau of Asian Research. Seatle, Washington 98105, USA

Murashige T. – Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plant. **15**. 473–497.

Nannipieri P. – Ascher J. – Ceccherini M. T. – Landi L. – Pietramellara G. – Renella G. (2003): Microbial diversity and soil functions. European Journal of Soil Science. **54**. 655–670.

Nassi N. – Angelini L. G. – Bonari E. (2010): Influence of fertilization and harvest time on fuel quality of giant reed (*Arundo donax* L.) in central Italy. European Journal of Agronomy. **32**. 3. 219–227. doi:10.1016/j.eja.2009.12.001

Nolte J. (1988): Pollution source analysis of river water and sewage sludge. Environmental Technology Letters. **9**. 8. 857–868. doi: 10.1080/09593338809384642

Nóvoa-Muñoz J. C. – Queijeiro J. M. G. – Blanco-Ward D. – Álvarez-Olleros C. – Martínez-Cortizas A. – García-Rodeja E. (2007): Total copper content and its distribution in acid vineyards soils developed from granitic rocks. The Science of the Total Environment. **378**. 1-2. 23–27. doi: 10.1016/j.scitotenv.2007.01.027

Nriagu J. O. (1979a): Copper in the atmosphere and precipitation. In: *Nriagu J.O.* (ed) Copper in the Environment, Part 1: Ecological Cycling. John Wiley & Sons, New York, pp. 45–75.

Nriagu J. O. (1979b): The global copper cycle. In: *Nriagu J. O.* (ed) Copper in the Environment, Part 1: Ecological Cycling. John Wiley & Sons, New York, pp. 1–17.

Nsanganwimana F. – Marchand L. – Douay F. – Mench M. (2014): *Arundo donax* L., a candidate for phytomanaging water and soils contaminated by trace elements and producing plant-based feedstock. A review. International Journal of Phytoremediation. **16**. 982–1017.

Oliver R. J. – Finch J. W. – Taylor G. (2009): Second generation bioenergy crops and climate change: a review of the effects of elevated atmospheric CO_2 and drought on water use and the implications for yield. Global Change Biology Bioenergy. **1**. 2. 97-114. doi: 10.1111/j.1757-1707.2009.01011.x

Page A. L. (Ed) (1982): Methods of soil analysis. Part 2: Chemical and microbiological properties (2nd ed.) American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin, USA.

Paoletti M. G. – Sommaggio D. – Petruzzelli G. – Pezzarossa B. – Barbafieri M. (1998): Earthworms as useful bioindicators of ecosystem sustainability in orchards and vineyards with different inputs. Applied Soil Ecology. **10**. 137-150.

Papazoglou E. G. – Karantounias G. A. – Vemmos S. N. – Bouranis D. L. (2005): Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. Environment International. **31.** 2243-249.

Paradelo M. – Arias-Estévez M. – Nóvoa-Muñoz J. C. – Pérez-Rodríguez P. – Torrado-Agrasar A. – López-Periago J. (2008): Simulating washoff of Cu-based fungicide sprays by using a rotating shear device. Journal of Agricultural Food Chemistry. **56**. 5795–8000.

Parat C. – Chaussod R. – Lévêque J. – Dousset S. – Andreux F. (2002): The relationship between copper accumulated in vineyard calcareous soils and soil organic matter and iron. European Journal of Soil Science. **53**. 663–669.

Pei-ying X. – Guo-xin L. – Wen-ju L. – Chang-zhou Y. (2010): Copper uptake and translocation in a submerged aquatic plant *Hydrilla verticillata* (L.f.) Royle. Chemosphere. **81**. 1098–1103. doi:10.1016/j.chemosphere.2010.09.023

Perdue R. E. (1958): *Arundo donax* – source of musical reeds and industrial cellulose. Economic Botany. **12**. 368–404.

Perlack R. D. et al. (2011): US Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry. Report No. ORNL/TM-2011/224 (US DOE, 2011).

Perwak J. – Bysshe S. – Goyer M. – Nelken L. – Scow K. (1980): Exposure and risk assessment for copper. Office of Water Regulation Standards, US Environmental Protection Agency, Washington, DC

Pietrzak U. – McPhail D. C. (2004): Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma. **122**. 151-166.

Ponizovsky A. A. – Allen H. E. – Ackerman A. J. (2007): Copper activity in soil solutions in calcareous soils. Environmental Pollution. **145**. 1–6.

Ponizovsky A. A. – Thakali S. – Allen H. E. – Di Toro D. M. – Ackerman A. J. (2006): Effect of soil properties on copper release in soil solution at low moisture content. Environmental Toxicology and Chemistry. **25**. 3. 671–682. doi: 10.1897/04-621R.1

Popp J. – Lakner Z. – Harangi-Rákos M. – Fári M. (2014): The effect of bioenergy expansion: Food, energy, and environment. Renewable and Sustainable Energy Reviews. **32**. 559–578. doi:10.1016/j.rser.2014.01.056

Porra R. J. – Thompson W. A. – Kriedemann P. E. (1989): Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta (BBA) Bioenergetics. **975**. 384-394.

Qianlai Z. – Zhangcai Q. – Min C. (2013): Biofuel, land and water: maize, switchgrass or *Miscanthus*? Environmental Research Letters. **8**. 015020 (6pp) doi:10.1088/1748-9326/8/1/015020

Rathmann R. – Szklo A. – Schaeffer R. (2010): Land use competition for production of food and liquid biofuels: an analysis of the arguments in the current debate. Renewable Energy. **35**. 14–22. http://dx.doi.org/10.1016/j.renene.2009.02.025.

Reeves R. D. – Baker A. J. M. (2000): Metal-accumulating plants. In: *Raskin I. – Ensley B. D.* (eds), Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment, Wiley, New York.

Reeves R. D. (2006): Hyperaccumulation of trace elements by plants. In: *Morel J.-L. – Echevarria G. –Goncharova N.* (eds), Phytoremediation of Metal-Contaminated Soils. Springer, NATO Science Series, Series IV: Earth and Environmental Sciences – Vol. 68. pp. 25–52.

Reichman S. M. (2002): The Responses of Plants to Metal Toxicity: a Review Focusing on Copper, Manganese and Zinc. Australian Minerals & Energy Environment Foundation, Melbourne.

REN21 (2012): Renewables 2012 Global Status Report. Renewable Energy Policy Network for the 21st Century. REN21 Secretariat, Paris, France.

RFA (2012): (Renewable Fuels Association) 2012 Historic US Fuel Ethanol Production (www.ethanolrfa.org/pages/statistics)

Richards B. K. – Stoof C. R. – Cary I. – Woodbury P. B. (2014): Reporting on marginal lands for bioenergy feedstock production — a modest proposal. Bioenergy Research. **7**. 3. 1060-1062. doi: 10.1007/s12155-014-9408-x

Robertson G. P. – Hamilton S. K. – Del Grosso S. J. – Parton W. J. (2011): The biogeochemistry of bioenergy landscapes: carbon, nitrogen, and water considerations. Ecological Applications. **21**. 4. 1055–1067. http://dx.doi.org/10.1890/09-0456.1

Rusjan D. – Strlič M. – Pucko D. – Korošec-Koruza Z. (2007): Copper accumulation regarding the soil characteristics in sub-Mediterranean vineyards in Slovenia. Geoderma. **141**. 111–118.

Rusjan D. (2012): Copper in Horticulture. In: *Dharumadurai D.* (ed) Fungicides for Plant and Animal Diseases. ISBN: 978-953-307-804-5, InTech, Available from: http://www.intechopen.com/books/fungicides-for-plant-and-animal-diseases/copper-in-horticulture.

Ruyters S. – Salaets P. – Oorts K. – Smolders E. (2013): Copper toxicity in soils under established vineyards in Europe: A survey. The Science of the Total Environment. **443**. 470–477. http://dx.doi.org/10.1016/j.scitotenv.2012.11.001.

Sagehashi M. – Liu C. – Fujii T. – Fujita H. – Sakai Y. – Hu H. – Sakoda A. (2011): Cadmium removal by the hydroponic culture of giant reed (*Arundo donax*) and its concentration in the plant. Journal of Water Environment Technology. **9**. 2. 121–127. http://doi.org/10.2965/jwet.2011.121

Sayen S. – Mallet J. – Guillon E. (2009): Aging effect on the copper sorption on a vineyard soil: column studies and SEM-EDS analysis. Journal of Colloid and Interface Science. **331**. 1. 47–54. doi: 10.1016/j.jcis.2008.11.049. Epub 2008 Nov 27.

Schlenker W. – Roberts M. J. (2009): Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change. Proceedings of the National Academy of Sciences of United States of America. **106**. 37. 15594–15598.

Schröder P. – Herzig R. – Bojinov B. et al. (2008): Bioenergy to save the world: producing novel energy plants for growth on abandoned land. Environmental Science and Pollution Research. **15**. 3. 196–204.

Schroeder H. A. – Nason A. P. – Tipton I. H. – Balassa J. J. (1966): Essential trace metals in man: copper. Journal of Chronic Diseases. **19**. 9. 1007–1034. doi:10.1016/0021-9681(66)90033-6

Schuler L. J. – Hoang T. C. (2008): Aquatic risk assessment of copper in freshwater and saltwater ecosystem of south Florida. Ecotoxicology. **17**. 642-659.

Scordia D. – Cosentino S. – Lee J. W. – Jeffries T. W. (2012): Bioconversion of giant reed (*Arundo donax* L) hemicellulose hydrolysate to ethanol by Scheffersomyces stipitis CBS6054. Biomass and Bioenergy. **39**. 296-305.

Searchinger T. – Heimlich R. – Houghton R. A. et al. (2008): Use of US croplands for biofuels increases greenhouse gases through emissions from land-use change. Science. **319**. 1238-1240.

Shabana K. – Qaisar M. – Iftikhar A. R. – Afsar K. – Sikandar S. – Mazhar A. G. – Shahida S. (2012): Potential of *Arundo donax* to treat chromium contamination. Ecological Engineering. **42**. 256–259. doi: 10.1016/j.ecoleng.2012.02.019

Shah K. – Kumar R. G. – Verma S. – Dubey R. S. (2001): Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. Plant Science. **161**. 1135–1144.

Shao H. – Chu L. (2008): Resource evaluation of typical energy plants and possible functional zone planning in China. Biomass and Bioenergy. **32**. 283–8.

Sharma K. P. – Kushwaha S. P. S. – Gopal B. (1998): A comparative study of stand structure and standing crops of two wetland species, *Arundo donax* and *Phragmites karka*, and primary production in *Arundo donax* with observations on the effect of clipping. Tropical Ecology. **39**. 1. 3–14.

Sharma S. K. – Sehkon N. S. – Deswal S. – John S. (2009): Transport and fate of copper in soils. World Academy of Science, Engineering and Technology. **3**. 3-22.

Sheldon A. R. – Menzies N. W. (2005): The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth.) in resin buffered solution culture. Plant and Soil. **278**. 341–349.

Shortall O. K. (2013): "Marginal land" for energy crops: exploring definitions and embedded assumptions. Energy Policy. **62**. 19-27. doi:10.1016/j.enpol.2013.07.048

Shu W. S. –Ye Z. H. – Lan C. Y. – Zhang Z. Q. – Wong M. H. (2002): Lead, zinc and copper accumulation and tolerance in populations of *Paspalum distichum* and *Cynodon dactylon*. Environmental Pollution. **120**. 445–453.

Simon L. – Koncz J. (2011): Accumulation of toxic metals from sewage sludge compost in *Salix viminalis* and *Arundo donax* energy plants. Proceedings of the 11th International Conference on Biogeochemistry of Trace Elements. Firenze, Italy, July 2011. Extended Abstracts. S1_77.pdf (CD-ROM).

Simon L. – Kovács B. – Barna S. – Varga C. – Dinya Z. (2009): Accumulation of heavy metals in *Arundo* and *Salix* energy plants treated with pig slurry, municipal sewage sludge and inorganic fertilizers. In: Proceedings of the 7th International Symposium on Trace Elements in Human: New Perspectives. Athens, Greece. 13-15 October, 2009. (ed. E. Pollet). University of Athens, Greece. CD-ROM. pp. 258-265.

Simon L. – Szabó B. – Szabó M. – Vincze G. – Varga C. – Uri Z. – Koncz J. (2013): Effect of various soil amendments on the mineral nutrition of *Salix viminalis* and *Arundo donax* energy crops. European Chemical Bulletin. **2**. 1. 18-21.

Singh P. K. – Tewari R. K. (2003): Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. Journal of Environmental Biology. **24**. 107-112.

Singh – Dharam K. – Nath –Sharma Y. K. (2007): Response of wheat seed germination and seedling growth under copper stress. Journal of Environmental Biology. **28**. 409-414.

Soler-Rovira P. – Fernández-Calviño D. – Arias-Estévez M. – Plaza C. – Polo A. (2013): Respiration parameters determined by the ISO-17155 method as potential indicators of copper pollution in vineyard soils after long-term fungicide treatment. The Science of the Total Environment. **447**. 25-31. doi.org/10.1016/j.scitotenv.2012.12.07.

Spatz H. C. H. – Beismann H. – Brüchert F. – Emanns A. – Speck T. (1997): Biomechanics of the giant reed *Arundo donax*. Philosophical Transactions of the Royal Society of London, B 352:1–10.

Stephenson A. L. – Dennis J. S. – Scott S. A. (2008): Improving the sustainability of the production of biodiesel from oilseed rape in the UK. Process Safety and Environmental Protection. **86**. 6. 427–440.

Stewart E. A. (1989): Chemical Analyses of Ecological Materials, 2ed. Blackwell Scientific Publications, Oxford, London, Edinburgh.

Strawn D. G. – Baker L. L. (2008): Speciation of Cu in a contaminated agricultural soil measured by XAFS, μ-XAFS, and μ-XRF. Environmental Science & Technology. **42**. 37–42.

Strawn D. G. – Baker L. L. (2009): Molecular characterization of copper in soils using X-ray absorption spectroscopy. Environmental Pollution. **157**. 2813–21.

Tang Y. – Xie J-S. – Geng S. (2010): Marginal land-based biomass energy production in China. Journal of Integrative Plant Biology. **52**. 1. 112–121.

Thapar R. – Srivastava A. K. – Bhargava P. – Mishra Y. – Rai L. C. (2008): Impact of different abiotic stress on growth, photosynthetic electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated *Anabaena doliolum*. Journal of Plant Physiology. **165**. 306-316.

Tilman D. et al (2009): Beneficial biofuels — the food, energy, and environment trilemma. Science. **325**. 5938. 270–271. doi*:* 10.1126/science.1177970

Toselli M. – Baldi E. – Marcolini G. – Malaguti D. – Quartieri M. – Sorrenti G. – Marangoni B. (2009): Response of potted grapevines to increasing soil copper concentration. Australian Journal of Grape Wine Research. **15**. 85-92.

Tucker G. C. (1990): The genera of Arundinoideae (Gramineae) in the southern United States. Journal Arnold Arboretum. **71**. 145–177.

Turnlund J. – Jacob R. – Keen C. – Strain J. J. – Kelley D. – Domek J. – Keyes W. – Ensunsa J. – Lykkesfeldt J. – Coulter J. (2004): Long-term high copper intake: effects on indexes of copper status, antioxidant status, and immune function in young men. American Journal of Clinical Nutrition. **79**. 1037–1044.

United States Congress (2007): Energy Independence and Security Act of 2007. Title II - Subtitle A - Renewable Fuel Standard. https://www.govtrack.us/congress/bills/110/hr6/text. Accessed 23 Dec 14

Upadhyay R. K. – Panda S. K. (2009): Copper-induced growth inhibition, oxidative stress and ultra-structural alterations in freshly grown water lettuce (*Pistia stratiotes* L.). Comptes Rendus Biologies. **332**. 7. 623–632. doi:10.1016/j.crvi.2009.03.001

USA (The United States of America) (2007): The Energy Independence and Security Act of 2007 (H.R. 6) (*www.gpo.gov/fdsys/pkg/BILLS-110hr6enr/pdf/BILLS-110hr6enr.pdf*)

Van Ranst E. – Verloo M. – Demeyer A. – Pauwels J. M. (1999): Manual for the soil chemistry and fertility laboratory. Ghent (Belgium): Ghent University, Faculty Agricultural and Applied Biological Sciences. p. 243.

Van Zwieten L. – Rust J. – Kingston T. – Merrington G. – Morris S. (2004): Influence of copper fungicide residues on occurrence of earthworms in avocado orchard soils. The Science of the Total Environment. **329**. 29–41.

Wang Q. (2005): Development and utilization of the energy plant. Journal of Fujian Forestry Science and Technology. **32**. 2. 1–5.

Wang Q. Y. – Zhou D. M. – Cang L. (2009): Microbial and enzyme properties of apple orchard soil as affected by long-term application of copper fungicide. Soil Biology & Biochemistry. **41**. 1504–1509. doi:10.1016/j.soilbio.2009.04.010.

Wang S. H. (2005): Different responses to Cu stress of different *Brassica juncea* L species. Journal of Biology. **22**. 30–32.

Wang T. A. (2005): Survey of the woody plant resources for biomass fuel oil in China. Science and Technology Review. **5**. 23. 12–4.

Wei X. R. – Hao M. D. – Shao M. A. (2007): Copper fertilizer effect on copper distribution and vertical transport in soils. Geoderma. **138**. 213–220.

Wightwick A. – Mollah M. – Partington D. – Allinson G. (2008): Copper fungicide residues in Australian vineyard soils. Journal of Agricultural Food Chemistry. **56**. 2457–2464.

Wightwick A. M. – Bui A. D. – Zhang P. – Rose G. – Allinson M. – Myers J. H. – Reichman S. M. – Menzies N. W. – Pettigrove V. – Allinson G. (2012): Environmental fate of fungicides in surface waters of a horticultural-production catchment in Southeastern Australia. Archives of Environmental Contamination and Toxicology. **62**. 3. 380–390. doi 10.1007/s00244-011-9710-y.

Wightwick A. R. – Walters G. – Allinson S. M. – Reichman – Menzies N. W. (2010): Environmental risks of fungicides used in horticultural production systems. In: *Carisse O.* (ed) Fungicides. InTech, Reijka, pp. 273–304.

Wu Qi Z. S. – Cheng L. – Wu G. (2012): Physiology and phytoaccumulation of *Arundo donax* subjected to exogenous copper. Acta Laser Biol Sintica. **21**. 4. 360–364. doi:10.3969 /j.issn.1007-7146.2012.04.013

Yruela I. (2005): Copper in plants. Brazilian Journal of Plant Physiology. **17**. 145–156. doi: org/10.1590/S1677-04202005000100012

Yu S. – He Z. L. – Huang C. Y. – Chen G. C. – Calvert D. V. (2004): Copper fractionation and extractability in two contaminated variable charge soils. Geoderma. **123**. 163–175. doi:10.1016/j.geoderma.2004.02.003.

Zengin F. K. – Kirbag S. (2007): Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings. Journal of Environmental Biology. **28**. 3. 561-566.

Zhang L. H. – Li P. J. – Li X. M. – Meng X. L. – Xu C. B. (2005): Effects of cadmium stress on the growth and physiological characteristics of wheat seedlings. Chinese Journal Ecology. **24**. 458–460.

Zhang Z. – Huang R. (2013): Analysis of malondialdehyde, chlorophyll, proline, soluble sugar, and glutathione content in Arabidopsis seedling. Bio-protocol Vol 3, Iss 14 http://www.bio-protocol.org/e817

Zhao S. – Liu Q. – Qi Y. – Duo L. (2010): Responses of root growth and protective enzymes to stress in turfgrass. Acta Biologica Cracoviensia Series Botanica. **52**. 2. 7-11.

Zhou L. X. – Wong J. W. C. (2003): Behavior of heavy metals in soils: effect of dissolved organic matter. In: *Selim H. M. – Kingery W. L.* (eds) Geochemical and Hydrological Reactivity of Heavy Metals in Soil. Lewis Publ., Chelse, MI. pp. 245-269.

11. PUBLICATIONS

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Subject: Ph.D. List of Publications

Candidate: Nevien Adel Elhawat
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List of publications related to the dissertation

Foreign language international book chapter(s) (4)

1. Alshaal, T., **Elhawat, N.**, Domokos-Szabolcsy, É., Kátai, J., Márton, L., Czakó, M., El-Ramady, H., Fári, M.G.: Giant Reed (Arundo donax L.): A Green Technology for Clean Environment.
   In: Phytoremediation: Management of Environmental Contaminants. Ed.: A.A. Ansari et al., Springer Science + Business Media, LLC, Switzerland, 3 - 20, 2015. ISBN: 9783319103945
   DOI: http://dx.doi.org/10.1007/978-3-319-10395-2_1

2. El-Ramady, H.R., Alshaal, T.A., Shehata, S.A., Domokos-Szabolcsy, É., **Elhawat, N.**, Prokisch, J., Fári, M., Márton, L.: Plant Nutrition:From Liquid Medium to Micro-farm.
   In: Sustainable Agriculture Reviews volume 14: Agroecology and Global Change. Ed.: Harry Ozier-Lafontaine, Magalie Lesueur-Jannoyer, Springer, Cham; Heidelberg; New York; Dordrecht; London, 449-508, 2014. ISBN: 9783319060156
   DOI: http://dx.doi.org/10.1007/978-3-319-06016-3

3. El-Ramady, H.R., Alshaal, T.A., Amer, M., Domokos-Szabolcsy, É., **Elhawat, N.**, Prokisch, J., Fári, M.: Soil Quality and Plant Nutrition.
   In: Sustainable agriculture aeviews volume 14: Agroecology and Global Change. Ed.: Harry Ozier-Lafontaine, Magalie Lesueur-Jannoyer, Springer, Cham; Heidelberg; New York; Dordrecht; London, 345-448, 2014. ISBN: 9783319060156
   DOI: http://dx.doi.org/10.1007/978-3-319-06016-3

4. Alshaal, T.A., El-Khir, A.M.A., **Elhawat, N.**, El-Essawi, T.M., El-Ramady, H.R.: Integrated nutrient management and soil quality.
   In: Contemporary environmental readings: towards sustainability for agriculture. Hassan R. El-Ramady, Neama A. Abd Alla, Said A. Shehata, LAP LAMBERT Academic Publishing, Germany, 1-121, 2013. ISBN: 9783659342905

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Foreign language scientific article(s) in Hungarian journal(s) (1)

5. **Elhawat, N.**, Alshaal, T., Kratz, S., Domokos-Szabolcsy, É., El-Ramady, H., Prokisch, J., Eszenyi, P., Sztrik, A., Babka, B., Fári, M.: Ecotoxicology of Copper in Horticultural Soils: A Review. *Int. J. Hortic. Sci. 19* (1-2), 7-18, 2013. ISSN: 1585-0404.

Foreign language scientific article(s) in international journal(s) (3)

6. **Elhawat, N.**, Alshaal, T., Domokos-Szabolcsy, É., El-Ramady, H., Márton, L., Czakó, M., Kátai, J., Balogh, P., Sztrik, A., Molnár, M., Popp, J., Fári, M.G.: Phytoaccumulation potentials of two biotechnologically propagated ecotypes of Arundo donax in copper-contaminated synthetic wastewater.
Environ. Sci. Pollut. Res. 21 (12), 7773-7780, 2014. ISSN: 0944-1344.
DOI: http://dx.doi.org/10.1007/s11356-014-2736-8
IF:2.757 (2013)

7. Alshaal, T., Domokos-Szabolcsy, É., Márton, L., Czakó, M., Kátai, J., Balogh, P., **Elhawat, N.**, El-Ramady, H., Gerőcs, A., Fári, M.: Restoring Soil Ecosystems and Biomass Production of Arundo donax L. under Microbial Communities-Depleted Soil.
Bioenerg. Res. 7 (1), 268-278, 2013. ISSN: 1939-1234.
DOI: http://dx.doi.org/10.1007/s12155-013-9369-5
IF:3.398

8. Alshaal, T., Domokos-Szabolcsy, É., Márton, L., Czakó, M., Kátai, J., Balogh, P., **Elhawat, N.**, El-Ramady, H., Fári, M.: Phytoremediation of bauxite-derived red mud by giant reed.
Environ. Chem. Lett. 11 (3), 295-302, 2013. ISSN: 1610-3653.
DOI: http://dx.doi.org/10.1007/s10311-013-0406-6
IF:1.906

Hungarian conference proceeding(s) (3)

9. **Elhawat N.**, Alshaal T., Domokos-Szabolcsy É., El-Ramady H., Antal G., Márton L., Czakó M., Balogh P., Fári M.: Biomassza termelés alternatív lehetősége rézzel szennyezett talajokon. In: XXI. Növénynemesítési Tudományos Napok : összefoglalók. Kiadta: a Magyar Tudományos Akadémia, Magyar Tudományos Akadémia, Martonvásár, 109, 2015.

10. Alshaal T., Domokos-Szabolcsy É., Márton L., Kátai J., **Elhawat N.**, Antal G., Gerőcsi A., Fári M.: Az olasznád (Arundo donax L.) szomatikus növények talaj mikrobiális közösségei és szerepük a fitoremediációs folyamatokban.
In: The proceeding of XIX. Növénynemesítési Tudományos Nap. Szerk.: Hoffmann Borbála, Kollaricsné Horváth Margit, Pannon Egyetem Georgikon Kar, Keszthely, 72, 2013.

11. **Elhawat N.**, Domokos-Szabolcsy É., Alshaal T., Molnár M., Antal G., Márton L., Fári M.: Szomatikus embrió eredetű olasznád (Arundo donax L.) klaszterek in vitro sótűrése két ökotípus összehasonlításával.
In: The proceeding of the XIX. Növénynemesítési Tudományos Nap : összefoglalók. Szerk.: Hoffmann Borbála, Kollaricsné Horváth Margit, Pannon Egyetem Georgikon Kar, Keszthely, 85, 2013. ISBN: 9789639639508

Foreign language conference proceeding(s) (9)

12. Alshaal, T., **Elhawat, N.**, El-Ramady, H., Domokos-Szabolcsy, É., Kátai, J., Márton, L., Czakó, M., Fári, M.: Influence of Giant Reed (Arundo donax L.) Plantation on Soil Quality in Different Regions: A Comparison Study.
In: Proceedings of the 11th International Conference "Climate Changes and Sustainable Development of Natural Resources. Ed.: by IUSS, International Union of Soil Sciences, Kafrelsheikh, Egypt, 80, 2014].

13. El-Ramady, H., Abd Alla, N., Alshaal, T., **Elhawat, N.**, Domokos-Szabolcsy, É., Prokisch, J., Sztrik, A., Fári, M.: Selenium: Its Implications to Human Health through Soils under Climate Changes.
In: Proceedings of the 11th International Conference "Climate Changes and Sustainable Development of Natural Resources. Ed.: by IUSS, International Union of Soil Sciences, Kafrelsheikh, Egypt, 54, 2014.

14. Domokos-Szabolcsy, É., El-Ramady, H.R., Alshaal, T., Márton, L., Zsiros, O., Garab, G., Veres, S., Prokisch, J., **Elhawat, N.**, Abd Alla, N., Fári, M.: Tolerance Efficiency and Soil Remediation Potential of Giant Reed (Arundo donax L.).
In: Proceedings of the 11th International Conference "Climate Changes and Sustainable Development of Natural Resources. Ed.: by IUSS, International Union of Soil Sciences, Kafrelsheikh, Egypt, 55, 2014.

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15. El-Ramady, H.R., Abd Alla, N.A., Alshaal, T.A., **Elhawat, N.**, Domokos-Szabolcsy, É., Prokisch, J.: Nano-selenium: From in vitro to micro farm experiments.
In: Proceedings of the International Conference on Biogeochemical processes at air-soil-water interfaces and environmental protection. Ed.: by ESSC, European Society for Soil Conservation, [Imola-Ravenna, Italy], 76, 2014.

16. Kurucz, E., Domokos-Szabolcsy, É., Antal, G., Kaprinyák, T., Alshaal, T., **Elhwat, N.**, Abd Alla, N., El-Ramady, H., Fári, M.: Biotechnology assisted breeding of endangered virginia mallow (Sida hermaphrodita L.) in Central Europe.
In: International Conference : Climate changes and sustainable development of natural resources : book of abstracts. Ed.: by Kafrelsheikh University, Kafrelsheikh University, Egypt, 81-82, 2014.

17. Fári, M., Antal, G., Kurucz, E., Kaprinyák, T., Alshaal, T., **Elhwat, N.**, Abd Alla, N., El-Ramady, H., Domokos-Szabolcsy, É.: Biotechnology of new dedicated biomass crops: Plantbiogen program in Hungary.
In: International Conference : Climate changes and sustainable development of natural resources : book of abstracts. Ed.: by Kafrelsheikh University, Kafrelsheikh University, Egypt, 22, 2014.

18. Fári, M.G., Antal, G., Alshaal, T., **Elhawat, N.**, El Ramady, H., Kurucz, E., Domokos-Szabolcsy, É.: Biotechnology of Biomass Supply Chain:Central European Perspective.
In: Proceedings of the Pannonian Plant Biotech Association Workshop "Plant for the future". Ed.: Pannonian Plant Biotech Association, 14-15,

19. **Elhawat, N.**, Alshaal, T., Domokos-Szabolcsy, É., Kátai, J., Márton, L., Czakó, M., Molnár, M., Fári, M.G.: Comparative performance of copper uptake by two ecotypes of giant reed (Arundo donax L.) grown on hydroponic culture.
In: Proceedings of the Pannonian Plant Biotech Association Workshop "Plant for the future". Ed.: by Pannonian Plant Biotech Association, Pannonian Plant Biotech Association, [Cluj-Napoca], 33-34, 2013.

20. Alshaal, T., **Elhawat, N.**, Domokos-Szabolcsy, É., Kátai, J., Márton, L., Czakó, M., Gerőcs, A., Fári, M.G.: Growth dynamics and biomass production of Giant Reed under different watering regimes.
In: Proceedings of the Pannonian Plant Biotech Association Workshop "Plant for the future". Ed.: by Pannonian Plant Biotech Association, Pannonian Plant Biotech Association, [Cluj-Napoca], 31-32, 2013.

List of other publications

Foreign language scientific article(s) in Hungarian journal(s) (4)

21. Shehata, S.A., Hashem, M.Y., Mahmoud, G.I., Abd El-Gawad, K.F., El-Ramady, H., Alshaal, T., Domokos-Szabolcsy, É., **Elhawat, N.**, Prokisch, J., Fári, M.: Effects of Foliar Nutrition on Onion Seed Storage under Controlled Atmosphere.
Int. J. Hortic. Sci. 19 (1-2), 101-110, 2013. ISSN: 1585-0404.

22. Shehata, S.A., Hashem, M.Y., Mahmoud, G.I., Abd El-Gawad, K.F., El-Ramady, H., Alshaal, T., Domokos-Szabolcsy, É., **Elhawat, N.**, Prokisch, J., Fári, M.: Effect of Foliar Nutrition on Post-Harvest of Onion Seed under Sandy Soil and Saline Irrigation Water Conditions.
Int. J. Hortic. Sci. 19 (1-2), 85-92, 2013. ISSN: 1585-0404.

23. Shehata, S.A., Hashem, M.Y., Mahmoud, G.I., Abd El-Gawad, K.F., El-Ramady, H., Alshaal, T., Domokos-Szabolcsy, É., **Elhawat, N.**, Prokisch, J., Fári, M.: Effects of Foliar Nutrition on Onion Seed Storage under Modified Atmosphere Packages.
Int. J. Hortic. Sci. 10 (1-2), 93-100, 2013. ISSN: 1585-0404.

24. Shehata, S.A., Hashem, M.Y., Mahmoud, G.I., Abd El-Gawad, K.F., El-Ramady, H., Alshaal, T., Domokos-Szabolcsy, É., **Elhawat, N.**, Prokisch, J., Fári, M.: Foliar Nutrition and Post-Harvest of Onion Seed: Effects of storage temperatures, storage period and foliar nutrition.
Int. J. Hortic. Sci. 19 (1-2), 29-47, 2013. ISSN: 1585-0404.

Foreign language scientific article(s) in international journal(s) (1)

25. El-Ramady, H., Abdalla, N., Alshaal, T., Domokos-Szabolcsy, É., **Elhawat, N.**, Prokisch, J., Sztrik, A., Fári, M., El-Marsafawy, S., Shams, M.S.: Selenium in soils under climate change, implication for human health.
Environ. Chem. Lett. 13 (1), 1-19, 2014. ISSN: 1610-3653.
DOI: http://dx.doi.org/10.1007/s10311-014-0480-4
IF:1.906 (2013)

Foreign language conference proceeding(s) (2)

26. Fári, M., Antal, G., Kurucz, E., Kaprinyák, T., Alshaal, T., **Elhwat, N.**, Abd Alla, N., El-Ramady, H., Domokos-Szabolcsy, É.: Bioipari célra nemesített évelő biomassza növények kutatása Debrecenben: Plantbiogen program = Research on dedicated perennial biomass crops in Debrecen : the plantbiogen program.
In: XX. Növénynemesítési Tudományos Nap : Növénynemesítés a megújuló mezőgazdaságban. Szerk.: Veisz Ottó, A Magyar Tudományos Akadémia Agrártudományok Osztályának Növénynemesítési Tudományos Bizottsága, [Budapest], 140-144, 2014. ISBN: 9789638351425

27. El-Ramady, H.R., Alshaal, T., Amer, M., Aiad, M., **Elhawat, N.**: Significance of nutrient and water sustainability: Effect of land leveling, cut off irrigation and N- fertilization on maize yield.
In: Proceedings of International Conference of German Soil Science Society (BDG). BDG, Göttingen, 1-4, 2013.

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STATEMENT

This thesis is made in the context of University of Debrecen Kerpely Kálmán Doctoral School, in order to obtain the University of Debrecen doctoral (Ph.D.) degree.

Debrecen, 20……………………

Signature of the candidate

STATEMENT

I certify that, …………………….…… the doctoral candidate between 20 ...... -20….. of the above mentioned Doctoral school, has worked under my supervision/ guidance of Doctoral School. Results included in this thesis are the candidate's self-forming activities, contributed to the thesis of the candidate independent work. I suggest/ recommend acceptance of the dissertation.

Debrecen, 20…………………

Signature of the supervisor

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