PHYSIOLOGICAL TOLERANCE OF PERENNIAL GRASSES TO HEAVY METAL CONTAMINATED SOILS

Barbara Rachele Ciaramella[1], Sebastiano Andrea Corinzia[1], Danilo Scordia[1], Cristina Patanè[2], Salvatore Luciano Cosentino[1], Giorgio Testa[1]*
[1]Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Università degli Studi di Catania, via Valdisavoia 5, 95123 Catania, Italy.
[2]CNR-Istituto per la BioEconomia (IBE), Sede Secondaria di Catania, Via P. Gaifami 18, 95126 Catania, Italy.
*dscordia@unict.it

ABSTRACT: There are several environmental advantages in the use of perennial crops such as reduction of soil erosion, increased soil organic matter, low fertilizer and agrochemical demands and increased biodiversity. Bioproducts, biofuels and bioenergy appears as an alternative to replace petroleum based products and nowadays, using land to grow crops for bioenergy has become an increasingly important policy objective designed in the RED II (2018/2001/EU). In this context, marginal lands appear as an alternative for industrial crops production without threatening food production. Marginal lands can be understood as generally as unproductivity lands which are exposed to stress conditions such as limitations of nutrients or water, or contamination by hydrocarbons or heavy metals. Therefore, production of biomass in marginal soils is being recommended to lessen land use change ethical issues linked with competition with food crops. Contaminated soils hinder the cultivation of traditional food crops, while industrial crops can provide several ecosystem services. However, understanding the tolerance and the phytoremediation of each species to a single contaminant helps us to better clean up the polluted areas and it can be useful for organizing protocols aimed at the reclamation of these areas, reducing time and reducing management costs providing a renewable source for energy and bioproducts. The species suitable to heavy metals polluted soils can be divided in two group: exclusion and resistance. The mechanisms of exclusion prevent the accumulation of toxic concentrations in sensitive sites within the cell, and thus preventing negative effects. The mechanisms of resistance generally concern the development of proteins that allow the plant to resist to heavy metals, allowing the accumulation of the same contaminant in the aerial parts of plant. The aim of this work was to study the physiological response of plants tolerant to heavy metals, in particular, focused the attention on two non-food lignocellulosic perennial grasses: giant reed (*Arundo donax* L.) and African fodder cane (*Saccharum spontaneum* L. ssp. *aegyptiacum*), in soils contaminated by four heavy metals (Cd, Pb, Ni, Zn) with the purpose to observe the physiological response and the mechanisms of resistance in the increasing concentrations of the four soil heavy metals.
Keywords: biomass, bioenergy, perennial grass, marginal land; dryness; water footprint; energy productivity.

1 INTRODUCTION

Lignocellulosic biomass can be used in various ways, such as an energy source, industrial feedstock and raw material in integrated biorefineries [1].

Nowadays, using land to grow crops for bioenergy has become an increasingly important policy objective designed in the RED II (2018/2001/EU), where Member States must supply a minimum of 14% of biofuels with at least 3.5% from advanced bioconversion by 2030. This target for transport biofuels is also contributing to a higher demand on biomass, which increases competition for land, threatening food security [2].

The use of agricultural land to cultivate energy crops has increased the competition between fuel and food. To avoid this problem, marginal lands appear as an alternative to energy crop production without threatening food production [3]. Marginal lands can be understood generally as unproductivity lands exposed to stressed conditions such as limitations of nutrients or water or contamination by hydrocarbons or heavy metals not suitable for food production. In heavy metals, there are several methods to decontaminate the soil through different paths using physical, chemical, or biological techniques. Naturally, phytoremediation is a sustainable and renewable technique that consists of utilizing the plants to remediate the contaminated site [4].

The selection of the plant needs to fulfill several conditions such as the tolerance of the crops to heavy metals, a high production of biomass, deep and extensive root systems, well know agronomic techniques, and low request for agronomic input. In this way, the cultivation of food crops in heavy metal contaminated soils must be avoided and the cultivation of industrial crops become a viable alternative, due both of their higher tolerance to the contaminant presence, which allows the crops growing without significant productivity losses, and their higher capability to accumulate the heavy metals, providing in this way, the remediation of the land [5,6].

Physiological and biochemical mechanisms to heavy metal tolerance have gained considerable insight during the last few decades. Plants employ specific strategies to tolerate harmful levels of heavy metals in the soil [7]. Most plants have developed a complicated process for the acquirements of relatively unavailable micronutrients like Zn, Mn, Cu, Fe, and Ni from the soil.

The mechanism of tolerance to metal stress in plants may range from the exclusion of toxic metal and inclusion and accumulation at inert places, with may vary from species to species [8].

Metal tolerance or accumulation can be enhanced by producing binding proteins and peptides in plants-high specificity of these peptides or proteins for more significance in plants [9].

However, tolerance levels in plants can be grouped into sensitive, resistant excluder, tolerant non-hyperaccumulator and hyper-tolerant hyperaccumulator species, each with specific physio-anatomical and molecular mechanisms for their resistance/tolerance to metal toxicity [11,12].

Moreover, the possibility of further utilizing biomass from phytoremediation turns energy crops in an excellent option for this technique and highlights the opportunity to enhance these areas that would be discarded with a consequent increase of environmental pollution [13]. In this scenario, *Arundo donax L.* and *Saccharum spontaneum L. ssp. aegyptiacum* are two perennial drought-tolerant grass species adapted to grow in marginal or sub-marginal lands, thus reducing competition with food crops for soil use, and the first results of experimental tests conducted in Sicily allow it to be indicated as a possible crop dedicated to the production of biomass for energy [14,15].

In this experiment, two lignocellulosic perennial grasses, *Arundo donax* L. and *Saccharum spontaneum* L. ssp. *aegyptiacum* were tested in contaminated soil with different concentrations of Zinc, Cadmium, Lead, and Nickel, seeking the physiological response and the mechanisms of resistance of these species to the heavy metals contaminated soils.

2 MATERIAL AND METHODS

2.1 Trial description

The present experiment was carried out during March-November 2020 at the University of Catania. The two lignocellulosic species, namely *Saccharum spontaneum* L. ssp. *aegyptiacum* (Willd.) and *Arundo donax* L. were grown in contaminated pot of 12 kg, previously contaminated with 4 heavy metal in two different levels, high and low. In particular, the soil was contaminated using nitrate of cadmium [$Cd(NO_3)_2$], nitrate of lead [$Pb(NO_3)_2$], nitrate of Zinc [$Zn(NO_3)_2$], and nitrate of nickel [$Ni(NO_3)_2$]. The concentration of heavy metal in the soil was applied followed EU guideline for contaminated soil, following as first concentration for each heavy metal the limit for contaminated soil, and as second concentration the double of the limit. The nitric metal salt per pot was calculated by multiplying the molecular weight of the salt by the grams of metal contained per pot and finally dividing by the molar weight of the metal. A nitrate fertilizer control was added to reach the same concentration of the highest level of $Zn(NO_3)_2$.

Clonal rhizomes of giant reed (*Arundo donax*) and African fodder cane (*Saccharum spontaneum* L. ssp. *aegyptiacum* (Willd.) were collected at the Experimental Farm of the University of Catania, Italy (10 m a.s.l., 37°25' N lat., 15° 03' E long.), weighted and size reduced as homogeneous as possible and transplanted in the pots two months after the soil contamination. The irrigation was kept in optimal conditions for the whole crop cycle. Pots were arranged in completely randomized design with 3 replications.

2.2 Measurements and calculations

During the growing season the leaf photosynthesis (A, μmol CO_2 m^{-2} s^{-1}), the transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and stomatal conductance (mol m^{-2} s^{-1}) were measured on three representative leaves in each species and treatment, on the third fully expanded leaf. A portable instrument (LCi-SD, ADC BioScientific, Great Amwell, Hertfordshire, UK) was used at a flow rate of 500 mL min^{-1} and under CO_2, temperature, and ambient humidity conditions during cloudless days.

The instantaneous water efficiency (iWUE) was calculated as the ratio between photosynthesis rate and leaf transpiration (μmol CO_2 mmol H_2O^{-1}).

2.3 Statistical analysis

The physiological data were statistically analyzed using R software (R Core Team, 2013). Species and pollutants were considered the main factors, and the means were separated by the Student-Newman-Keuls (SNK) test at a 95% confidence level. The mechanisms of resistance to increasing soil pollutant concentration of giant reed and African fodder cane were evaluated through linear relationships between the pollutant concentration and stomatal conductance, with the b-value of the linear regression representing the plant response to increasing heavy metal contentration. The Shapiro-Wilk test was used to verify the normality of the residual distribution. Regression coefficients were considered significant when $P \leq 0.05$, and the goodness of fit was estimated by calculating the R^2 (SigmaPlot11, Systat Software Inc., San Jose, CA, USA).

3 RESULTS

3.1 Photosynthesis rate

The ANOVA showed significant differences concerning the photosynthesis rate compared with the treatments (Table 1). The date of the measurement was significant for Cd, Pb, and Ni but not for Zn. The species showed significant differences for Ni and Pb but not for Cd and Zn. Pollutant concentration and first and second-order interactions did not show significant differences in photosynthesis rate.

Table I: ANOVA of the main factors and interactions for the net photosynthesis of *Arundo donax L.* and *Saccharum spontaneum spp. aegyptiacum* under increasing levels of pollutants in the soil (significance level at $P \leq 0.05$).

Photosynthesis rate	**Cd**	**Ni**	**Pb**	**Zn**
Date **(D)**	0.019	0.000	0.001	0.061
Genotypes **(G)**	0.128	0.000	0.002	0.260
Concentration **(C)**	0.736	0.226	0.937	0.988
D x G	0.071	0.387	0.628	0.063
D x C	0.736	0.948	0.748	0.411
G x C	0.792	0.994	0.522	0.180
D x G x C	0.125	0.946	0.557	0.738

Across the average of measurements, *Saccharum* showed a higher photosynthesis rate than giant reed under the control and the different levels of contaminants used in the study (Fig 1).

In the cadmium-contaminated theses, the photosynthesis rate in *Saccharum* did not show significant differences through cadmium concentration equal to 4 mg kg^{-1} of soil and cadmium concentration equal to 8 mg kg^{-1} of soil, although values were lower than the controls. *Arundo* slightly decreased photosynthetic rate under Cd contamination but at the highest level as compared with the control (12 at Cd8 and 14.5 μmol CO_2 m^{-2} s^{-1}, respectively). In the nickel-contaminated both species showed a decreasing photosynthetic rate with the increasing concentrations of the metal being studied. And in particular, the lower values were recorded in the Ni 220 mg kg^{-1} in the soil in both species (13 and 11 μmol CO_2 m^{-2} s^{-1}, respectively for *Saccharum* and *Arundo*). In the lead-contaminated, *Saccharum* showed greater sensitivity to this heavy metal, obteined lower value in the photosynthesis rate, in particular with the highest level of lead in the soil (Pb900). *Arundo* showed lower photosynthetic rate values than the controls, but no significant differences were found between the two concentration levels of contaminant in the soil (average Pb levels, 11 µmol CO_2 m^{-2} s^{-1}). Finally, photosynthesis rate was always lower than the controls for both specie in soil contaminated with zinc, but between the different levels of contaminant present in the soil, it was higher for *Saccharum* in the level of 450 mg kg^{-1} of zinc and lower in the concentration of 900 mg kg^{-1} of zinc in the soil (14 and 12 µmol CO2 m^{-2} s^{-1}, respectively). Surprisingly *Arundo* increased leaf photosynthesis at 900 mg Zn kg^{-1} than in the concentration 450 mg kg^{-1} (10 and 13 µmol CO_2 m^{-2} s^{-1}, respectively).

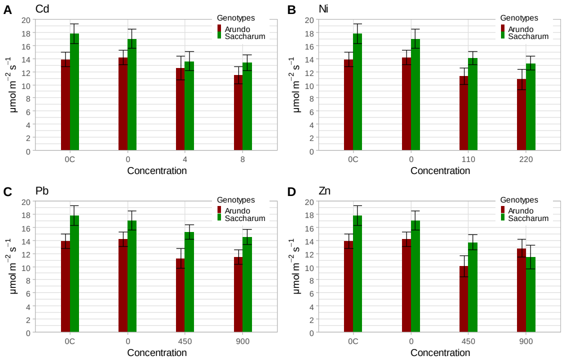

Figure 1: Leaf photosynthesis (µmol CO_2 m^{-2} s^{-1}) in *Arundo donax* and *Saccharum spontaneum spp. aegyptiacum* under different levels of contaminant (A: Cd, B: Ni, C: Pb; D: Zn) and control unfertilized (0) and control N fertilized (0C).

3.2 Transpiration rate

The ANOVA showed significant differences of main effect on the transpiration rate (Tab. 2).

Table II: ANOVA of the main factors and interactions for the leaf transpiration rate of *Arundo donax* L. and *Saccharum spontaneum spp. aegyptiacum* under increasing levels of pollutants in the soil (significance level at P≤0.05).

Traspiration rate	**Cd**	**Ni**	**Pb**	**Zn**
Date **(D)**	0.006	0.571	0.084	0.214
Genotype **(G)**	0.002	0.000	0.000	0.285
Concentration (C)	0.882	0.960	0.531	0.444
D x G	0.042	0.037	0.007	0.222
D x C	0.758	0.708	0.554	0.637
G x C	0.414	0.531	0.195	0.288
D x G x C	0.039	0.478	0.128	0.908

The date of the measurement was only significant for Cd. The genotype showed substantial differences for Cd, Ni, and Pb, while the pollutant concentration showed no differences. The data x genotype interactions were significantly different for Cd and Ni and the second-order interaction only for Cd. The two species under study showed a distinct tendency to transpiration depending on the contaminants present in the soil and their concentration, similarly to the leaf photosynthesis. In *Arundo*, the transpiration rate detected was higher than in *Sacchurum* in accordance with the photosynthetic cycle of the crop, C4 in *Saccharum* and C3 in *Arundo* (Fig. 2).

The higher transpiration rate was obtained among the controls in all treatments, and in particular, the fertilized one had higher transpiration rate than the unfertilized control. Among the cadmium – contaminant, similarly to the photosynthesis rate, the highest transpiration rate between the two levels of contaminant in the soil was found for *Saccharum* in the lower cadmium concentration equal to 4 mg kg^{-1}; for *Arundo* no significant differences were found in the transpiration rate in both contaminant levels. Among the nickel-contaminated, the transpiration rate was lower than the controls. In *Arundo*, the transpiration rate decreased when the concentration of nickel in the soil increased. In *Saccharum*, although not significant, the transpiration rate increased in the highest concentration of nickel in the soil (220 mg kg^{-1}). Among the lead-contaminated trials, both *Arundo* and *Saccharum* showed a higher transpiration rate in the controls than the two levels of lead present in the soil. Between the two contaminant levels, *Arundo* did not show significant differences (3.7 mmol H_2O m^{-2} s^{-1} in the average of concentration), *Saccharum* instead showed a higher transpiration rate in the trial containing 450 mg kg^{-1} lead in soil and lower in 900 mg kg^{-1} of lead in soil (2.9 and 2.5 mmol H_20 m^{-2} s^{-1}, respectively). Finally, for treatments contaminated by zinc, the species showed a different trend within the levels of the heavy metal present in the soil. *Arundo* showed an increasing transpiration rate at the higher contaminant level (4 mmol H_20 m^{-2} s^{-1} to 900 mg kg^{-1} of zinc in soil versus 3.24 mmol H_20 m^{-2} s^{-1} at 450 mg kg^{-1} of zinc in the soil). *Saccharum* showed a decreasing transpiration rate in the level with the highest concentration of zinc in the soil (2.9 mmol H_20 m^{-2} s^{-1} at 900 mg kg^{-1} of zinc in the soil).

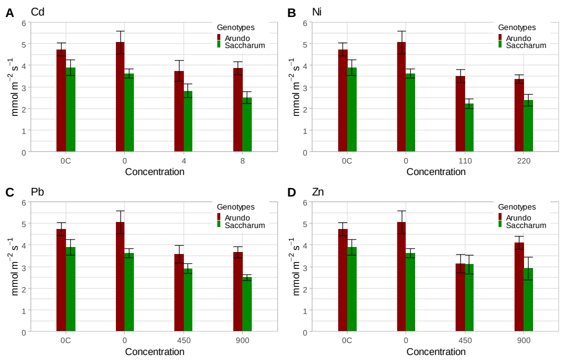

Figure 2: Leaf transpiration rate of *Arundo donax* L. and *Saccharum spontaneum spp. Aegyptiacum* in the different levels of contaminant (A: Cd, B: Ni, C: Pb; D: Zn) and control unfertilized (0) and control N fertilized (0C).

3.3 Stomatal conductance

The ANOVA showed significant differences concerning the stomatal conductance of the treatments under study (Tab. 3). The measurement date, the genotype, and the date x genotype interaction were significant in all the pollutants studied. The pollutant concentration and the first and second-order interactions did not show differences between the various pollutants. The stomatal conductance, in both species and contaminants was similar to the transpiration rate and the photosynthesis rate, obtaining higher values in the controls pots than in the contaminated pots for both species. Stomatal conductance was consistently lower in *Saccharum* due to the C4 photosynthetic cycle, as discussed in the transpiration rate (Fig. 3). The trend of stomatal conductance in the *Arundo* showed higher values in the controls, in particular was also higher in the non-fertilized control compared to the fertilized one (0.145 and 0.132 mol m^{-2} s^{-1}, respectively).

Table III: ANOVA of the main factors and interactions for the stomatal conductance of *Arundo donax* L. and *Saccharum spontaneum spp. aegyptiacum* under increasing levels of pollutants in the soil (significance level at P≤0.05).

Stomatal conductance	**Cd**	**Ni**	**Pb**	**Zn**
Date **(D)**	0.000	0.000	0.000	0.003
Genotype **(G)**	0.000	0.000	0.000	0.030
Concentration (C)	0.649	0.769	0.395	0.414
D x G	0.000	0.004	0.007	0.034
D x C	0.669	0.882	0.345	0.653
G x C	0.397	0.817	0.691	0.501
D x G x C	0.086	0.737	0.265	0.989

The stomatal conductance in *Arundo* was higher in the pots with the higher levels of the metals, and in particular in cadmium with 8 mg kg^{-1} and in zinc 900 mg kg^{-1}. Moreover, in the two levels of nickel and lead, the highest conductance values were recorded in the lower level of contaminant, and in particular at the concentration of 110 mg kg^{-1} of nickel in the soil (0.098 mol m^{-2} s^{-1}) and the concentration of 450 mg kg^{-1} of lead in the soil (0.091 mol m^{-2} s^{-1}).

The trend of stomatal conductance in *Saccharum* was higher in the controls, and in particular, the fertilized one showed a higher stomatal conductance than the non-fertilized controls (0.090 and 0.075 mol m^{-2} s^{-1}, respectively). In the pots contaminated by cadmium, lead, and zinc in relation with the two concentrations of heavy metal present in the soil, *Saccharum* showed higher values of stomatal conductance in the low level of the heavy metal concentration. Except for the two levels of nickel concentration, where *Saccharum* does not show significant differences.

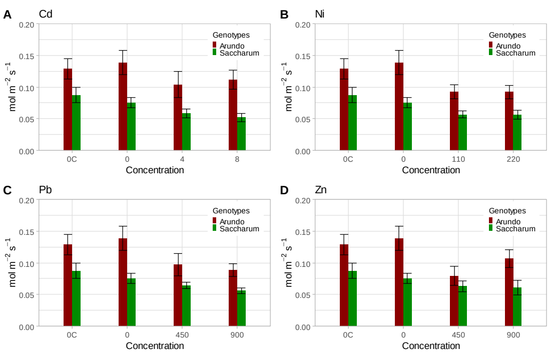

Figure 3: Stomatal conductance of *Arundo donax* L. and *Saccharum spontaneum spp. aegyptiacum* in the different levels of contaminant (A: Cd, B: Ni, C: Pb; D: Zn) and control unfertilized (0) and control N fertilized (0C).

3.4 Instantaneous water used efficiency

The ANOVA showed significant differences concerning the instantaneous water used efficiency (iWUE) of the treatments under study (Tab. 4). The measurement data was significant for Ni and Pb, the genotype for all pollutants, while the concentration and the interactions of the concentration with the other factors were not significant. The data x genotype interactions showed differences only for Ni and Pb.

Table IV: ANOVA of the main factors and interactions for the instantaneous water use efficiency of *Arundo donax* and *Saccharum spontaneum spp. aegyptiacum* under increasing levels of pollutants in the soil (significance level at P≤0.05).

iWUE	**Cd**	**Ni**	**Pb**	**Zn**
Date **(D)**	0.506	0.000	0.000	0.291
Genotype **(G)**	0.001	0.000	0.000	0.000
Concentration (C)	0.926	0.115	0.463	0.377
D x G	0.974	0.005	0.003	0.913
D x C	0.758	0.795	0.88	0.469
G x C	0.163	0.251	0.467	0.640
D x G x C	0.495	0.32	0.342	0.431

The iWUE, which represents the ratio between the photosynthesis and the transpiration rate expresses the micromoles of CO_2 absorbed on the mole of transpiration H_2O, showed lower levels in the controls compared to the all polluted pots (Fig. 4). Among the species, *Saccharum* always showed higher iWUE values than *Arundo*, both in the controls and in the various levels of soil contaminant, by its C4 photosynthetic cycle. For cadmium, the highest iWUE value was obtained at the concentration of 8 mg kg^{-1} of soil in *Saccharum*, a value much higher than that of the controls (6.0 and 4.5 µmol CO_2 mmol H_2O, respectively). For *Arundo* the higher value was obtained at the concentration of cadmium equal to 4 mg kg^{-1} (4.0 µmol CO_2 mmol H_2O). For nickel, the highest iWUE values were obtained in *Saccharum*, and in particular, the highest value was obtained at the nickel concentration equal to 110 mg kg^{-1}, followed by the concentration of 220 mg kg^{-1} of nickel in the soil (7.0 and 6.5 µmol CO_2 mmol H_2O, respectively). For *Arundo*, no significant differences were observed between the controls and metal concentrations present in the soil (3.0 µmol CO_2 mmol H_2O, on average). In the lead treatment, the higher values of iWUE were found in Saccharum at a concentration of 900 mg kg^{-1} of lead in the soil (5.8 µmol CO_2 mmol H_2O^{-1}), while for *Arundo*, no significant differences were observed between study concentrations and controls (3.2 µmol CO_2 mmol H_2O^{-1}, on average). Finally, for zinc, both *Saccharum* and *Arundo* did not show significant differences between the different controls and the different contaminant levels, reaching average values of about 4.5 µmol CO_2 mmol H_2O^{-1} for *Saccharum* and 3.0 µmol CO_2 mmol H_2O^{-1} for *Arundo*.

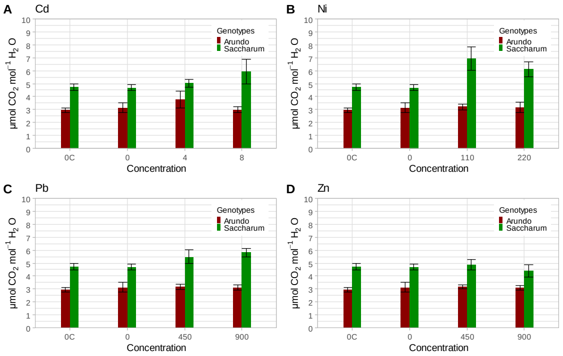

Figure 4: Instantaneous WUE of *Arundo donax* and *Saccharum spontaneum spp. Aegyptiacum* at different levels of contaminant (A: Cd, B: Ni, C: Pb; D: Zn) and control unfertilized (0) and control N fertilized (0C).

3.5 Mechanisms of resistance

The relationships between soil contaminants at different levels and the stomatal conductance of *Arundo donax* and *Saccharum spontaneum spp. aegyptiacum* was calculated on the average of the measurements made during the crop cycle and highlighted the different mechanisms of the species under study in response to heavy metal pollution of the soil (Fig. 5).

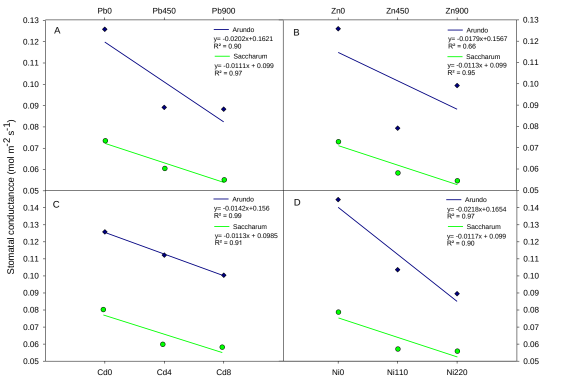

Figure 5: Linear relationships between contaminated soil and stomatal conductance (mol m^{-2} s^{-1}) in *Arundo donax* and *Saccharum spontaneum* spp. *aegyptiacum* in the different contamination levels (A: Cd, B: Ni, C: Pb; D: Zn).

In general, there was a linear and decreasing trend of stomatal conductance in all the relationships considered as the concentration of contaminated soil increases.

Saccharum showed a lower stomatal conductance in all relations than the Arundo, and in particular, the slope and intercept values of the linear relations were consistently lower, indicating a greater tolerance response. Considering the value of the slope, *Arundo* showed a decrease of 0.0202 mol m^{-2} s^{-1} with increasing Pb concentration with 90% adaptation of the estimated data compared to those observed, against a decrease of 0.0111 mol m^{-2} s^{-1} in Saccharum (R^2 = 0.97). In the soil contaminated with Zn, the *Arundo* showed a decrease of 0.0179 mol m^{-2} s^{-1} in stomatal conductance with increasing Zn concentration (R^2=0.66), against a decrease of 0.0113 mol m^{-2} s^{-1} was observed in *Saccharum* (R^2 = 0.95). In the case of soil contamination with Cd, the slope in *Arundo* reduced as the concentration of Cd increases (0.0142 mol m^{-2} s^{-1}) with an approximation of 99% and was relatively similar to Saccharum, which however recorded values similar to the previous relationships described (0.0113 mol m^{-2} s^{-1} and R^2 = 0.91). In the relationship between Ni concentration and stomatal conductance, the *Saccharum* slightly increased the slope value (0.0117 mol m^{-2} s^{-1}; R^2 = 0.90), while the Arundo showed the highest slope value as compared to the other relationships (0.0218 mol m^{-2} s^{-1}; R^2 = 0.97)

4 DISCUSSION

Arundo donax and *Saccharum spontaneum spp. aegyptiacum*, showed characteristics of physiological resistance in soils polluted by heavy metals (Cd, Pb, Ni and Zn). With the development and the transition to the bio-economy sectors will mainly rely on the availability of sustainable biomass, in terms of yield per unit area and quality of raw material, on competition for land, food, resources and development of new biotechnologies [15]. In this context, the crops studied could suitable for the supply of raw material without competing for land, food and resources with food crops and capable of enhancing land polluted by heavy metals that would otherwise be abandoned, with consequent risk for the surrounding ecosystem.

The studied species showed different physiological responses to soil contaminants. In particular, *Saccharum spontaneum spp. aegyptiacum*, a bioenergy grass with a photosynthetic cycle C4, showed a higher photosynthesis a lower stomatal conductance and transpiration rates as compared to the C3 *Arundo donax* [16] and other C3 crops [17], thus showing an increased efficiency in the use of resources (mainly solar radiation and water available soil) in all conditions of heavy metals in the soil.

The linear relationships between the increasing levels of soil pollutants and the stomatal conductance, provides an idea of the mechanism to cope with stress adopted. In general, in all the relationships considered, there was a linear and decreasing trend of the stomatal conductance when the concentration of contaminated soil increases, indicating that the plants suffer gradually increasing the contaminant, tending so to close the stomata. The *Saccharum* showed in all the calculated relationships a lower stomatal conductance than the *Arundo* (highlighted by the lower values of the intercept), and in particular a response contained in the reduction of the stomatal conductance while the concentration of soil pollutant increases (values lower than the slope), indicating a good ability to tolerate the experimental conditions imposed in the present work.

The *Arundo*, while undergoing greater stress in the higher level of pollutants in the soil, indicated by a greater reduction in stomatal conductance, continued to maintain a certain stomatal opening thus ensuring normal gas exchanges with the atmosphere and consequently allowing to accumulate dry matter in biomass thanks to the photosynthetic process [16, 18].

5 CONCLUSION

In conclusion, the present study highlighted the ability of the two biomass species, no-food lignocellulosic perennial grasses to tolerate the heavy metals in the soil, and the physiological responses to increasing stress. The relationships between the increasing levels of soil pollutants and the stomatal conductance provide an insight of the stress condition of the plant. The linear and negative response of stomatal conductance to increasing soil contaminants, indicated that the plants which are subject to increasing levels of soil pollutant suffer gradually increasing stress, tending to close the stomata. This is one of mechanism of resistance that the plant adopted, showing the tolerance to the heavy metal of the two species. The smaller "b value" in all linear regressions suggests *Saccharum* more tolerant than *Arundo* to increasing soil contaminants. However, present physiological results must be confirmed when morphological and productive data will be elaborated.

5 ACKNOWLEDGEMENTS

This paper is part of a project that has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 727698, project MAGIC (Marginal lands for growing industrial crops: turning a burden into an opportunity.

6 REFERENCES

[1] Scordia, D., Cosentino, S.L., 2019. Perennial Energy Grasses: Resilient Crops in a Changing European Agriculture. Agriculture, 9(8), 169.

[2] EU DIRECTIVE (EU) 2018/2001 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 11 December 2018 on the promotion of the use of energy from renewable sources (recast), 2018.

[3] Cosentino, S.L., Copani, V., Testa, G., Scordia, D., 2015. Saccharum spontaneum L. ssp. aegyptiacum (Willd.) Hack. a potential perennial grass for biomass production in marginal land in semi-arid Mediterranean environment. Industrial Crops and Products, 75, 93-102.

[4] Barbosa, B., Boléo, S., Sidella, S., Costa, J., Duarte, M.P., Mendes, B., Cosentino S.L., Fernando, A.L., 2015. Phytoremediation of Heavy Metal-Contaminated Soils Using the Perennial Energy Crops Miscanthus spp. and Arundo donax L, Bioenergy Res. 8 (2015) 1500–1511.

[5] 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. Environmental International, 31:243–249.

[6] Nanda Kumar, P.B.A., Dushenkov, V., Motto, H., Raskin, I., 1995. Phytoextraction: The Use of Plants to Remove Heavy Metals from Soils, Environ. Sci. Technol. 29 (1995) 1232–1238.

[7] Liu, L., Li, W., Song, W., Guo, M., 2018. Remediation techniques for heavy metal-contaminated soils: Principles and applicability, Sci. Total Environ. 633, 206–219.

[8] Raskin, I., Ensley, B., 2000. Phytoremediation of toxic metals using plants. Wiley, Canada, p 471.

[9] Ryu, S.K., Park, J.S., Lee, I.S., 2003. Purification and characterization of a copper binding protein from Asian periwinkle Littorina brevicula. Comp Biochem Physiol C Toxicol Pharmacol 134:101-107.

[10] Öztürk M., Ashraf M., Aksoy A., Ahmad, M.S.A. (Eds.) Phytoremediation for Green Energy, 2015. Springer. ISBN: 978-94-007-7886-3.

[11] Alloway BJ, 1995. Soil processes and the behaviour of metals. In: Alloway B.J. (Ed), Heavy metals in soils (pp. 38–57). London: Blackie.

[13] Scordia, D., Testa, G., Cosentino, S.L., 2014. Perennial grasses as lignocellulosic feedstock for second-generation bioethanol production in Mediterranean environment. Italian Journal of Agronomy 9 (581), 84–92.

[14] Scordia, D., Testa, G., Copani, V., Patanè, C., Cosentino, S.L., 2017. Lignocellulosic biomass production of Mediterranean wild accessions (Oryzopsis miliacea, Cymbopogon hirtus, Sorghum halepense and Saccharum spontaneum) in a semi-arid environment. Field Crops Research, 214, 56-65.

[15] Cosentino, S.L., Testa, G., Scordia, D., Alexopoulou, E., 2012. Future yields assessment of bioenergy crops in relation to climate change and technological development in Europe. Italian Journal of Agronomy, 7, 22.

[16] Cosentino, S.L., Patanè, C., Sanzone, E., Testa, G., Scordia, D., 2016. Leaf gas exchange, water status and radiation use efficiency of giant reed (Arundo donax L.) in a changing soil nitrogen fertilization and soil water availability in a semi-arid Mediterranean area. European Journal of Agronomy, 72, 56–69

[17] Cosentino, S.L., Riggi, E., Testa, G., Scordia, D., Copani, V., 2013. Evaluation of European developed fibre hemp genotypes (Cannabis sativa L.) in semi-arid Mediterranean environment. Industrial Crops and Products, 50, 312– 324

[18] Cosentino, S.L., Scordia, D., Sanzone, E., Testa, G., Copani, V., 2014. Response of giant reed (Arundo donax L.) to nitrogen fertilization and soil water availability in semi-arid Mediterranean environment. European Journal of Agronomy 60, 22–32.