 doi:10.20944/preprints201612.0014.v1

Peer-reviewed version available at *Sustainability* **2017**, *9*, 81; doi:10.3390/su9010081

Article

Germination of *Bouteloua dactyloides* and *Cynodon dactylon* in a Multi-Polluted Soil

María Del Rosario Delgado-Caballero [1], María Teresa Alarcón-Herrera [1], María Cecilia Valles-Aragón [2,*], Alicia Melgoza-Castillo [3], Arwell Leyva-Chávez [2] and Damaris Lepoldina Ojeda-Barrios [2]

[1] Advanced Materials Research Center (CIMAV), Durango 34000, Dgo., Mexico; rosariodelgado369@gmail.com (M.D.R.D.-C.); teresa.alarcon@cimav.edu.mx (M.T.A.H.)
[2] Faculty of Agrotechnological Science, Autonomous University of Chihuahua, Chihuahua 31110, Chih., Mexico; nleyva@uach.mx (A.L.-C.); dojeda02@hotmail.com (D.L.O.-B.)
[3] Faculty of Zootechnics and Ecology, Autonomous University of Chihuahua, Chihuahua 31110, Chih., Mexico; amelgoza@uach.mx
* Correspondence: valles.cecilia@gmail.com; Tel.: +52-614-439-1844

Abstract: Mining wastes generate a high environmental impact, and population exposure to metals and metalloids. Phytoremediation is a technology that uses plants to remediate polluted sites, one of its limitations is seed germination in soil with high content of metals and metalloids. *B. dactyloides* is a native specie from semiarid regions, while *C. dactylon* is an introduced specie, both are tolerant to drought and low temperatures. Owing, the objective of this research was to evaluate the germination of both species, exposed to a multi-polluted soil with As, Cd, Pb and Zn of a mining site, pondering different pH conditions (from 5.0 to 9.0). The study considered 4 repetitions by type of seed and soil pH. *B. dactyloides* showed higher germination percentage (83%) with pH 7.8; while the greater germination of *C. dactylon* was 34 % at pH 6.0. In the soil at pH 5, the germination in both species was not reached, owing that metals are more bioavailable in an acid environment. *C. dactylon* is less tolerant to As, Cd, Pb and Zn, so it is considered not effective for phytoremediation process. *B. dactyloides* has a high phytoremediation potential for multi-polluted sites, but further experiments are needed.

Keywords: sustainable technology; phytoremediation; germination; *Bouteloua dactyloides*; *Cynodon dactylon*

---

1. Introduction

The wastes generated by mining are a high-impact environmental problem because population can be exposed to metals and metalloids. The change of pH and oxidation state can increase or decrease the potential bioavailability of metals in soil [1]. Metals such as Zn, Cu, and Mn are essential for living organisms at low concentrations, but become toxic at increasing concentrations. Other metals like Hg, Pb and Cd have never been shown to be essential for organism development and are toxic at low concentrations [2]. Contamination of the environment with heavy metals by the increased industrialization and geochemical activities is a major environmental and human health problem [3], due to their non-biodegradability and high toxicity [4].

Potentially toxic elements (PTE) in Mexico, such as Pb, Cd, Zn, As, Se, and Hg, are commonly derived from mining processes [5]. From these elements Pb and Cd are the polluters most frequently found in the mining zones of the country [6]. Moreover, in soil the metals of most concern are Cd and Zn, as they show the greatest mobility [3]. The environmental impact caused by the pollution in mining sites depends on the interaction capacity of metals with soil and water [1]. The degree of metal toxicity hangs on the element and its bioavailability, that it is control by abiotic factors such as metal concentration, soil pH, and biotic factors such as the presence of metal-liberating microflora [2]. If the

Peer-reviewed version available at Sustainability 2017, 9, 81; doi:10.3390/su9010081

metals are bioavailable in the soil, can affect the fertility and/or later land use, as well as induce the population exposure [7].

Phytoremediation is an emerging technology that uses plants and their rhizosphere to extract, detoxify, and sequester pollutants (organic and inorganic) from soil, sediments and water [8]. This technology it is considered a higher cost-benefit alternative compared to mechanic or physic-chemical processes [9]. The pollutant uptake by plants depends on pH, organic substances, metal content, and other elements in the rhizosphere [2]. Seed germination is the first step of the plant life and it is one of the most sensitive process in the plants physiology, affected by hormonal interactions and environmental factors, both biotic and abiotic, and therefore of the metals presence [10]. The use of seeds for phytoremediation processes is a source to determine if plants are capable of growing in polluted soil [8]. Unfortunately, predicting the response of organisms simultaneously exposed to more than one PTE is a difficult task in environmental toxicology [4].

B. dactyloides, which is commonly named as "Buffalograss", is a low profile gramínea, native from semi-arid regions in Mexico. Buffalograss is a perennial cycle plant that requires low amounts of water, is drought resistant, tolerates high salinity and low temperatures, has a low nutrient demand, and grows in argillaceous soil [11]. *C. dactylon*, commonly known as "Bermudagrass", is native plant from Africa, which has perennial cycle, grows from argillaceous to sandy soils with pH of 5.5 to 5.7, tolerates salinity, dry, and warm summers. Both species germinate in a temperature range from 20 to 35 °C [12]. Due to the high tolerance to arid conditions of both species *B. dactyloides* and *C. dactylon*, the objective of this research was to evaluate their germination exposed at multi-polluted soil with As, Cd, Pb and Zn of a mining site, pondering different soil pH conditions.

2. Materials and Methods

2.1. Soil collection

The collect soil was conducted at the southwest of Chihuahua City, Mexico, in an area with mining residues (tailing dams) from a former foundry. The universal coordinates which were registered the sampling area were X=402288.6431, Y=3166594.5405, in #13 zone.

5 different soil were collected superficially (at 5 cm) and extracted with a stainless steel shovel, and classified them as A, B, C, D and E. Also, a reference target of non-polluted soil with similar texture characteristics that polluted soils named F was collected.

2.2. Soil pH

The pH determination of the soil was done to each collected soil (A, B, C, D, E, F) with Thermo ORION 3 STAR equipment, previously calibrated. The samples were prepared according to the method from the mexican regulation NOM-147 [13].

The pH of D soil was chemically modified to preserve strongly acid conditions, adding 200 mL of hydrochloric acid (HCl) 3N to 500 g of soil.

Soil E was modified to preserve strongly alkaline conditions (pH 9). To 500 g of soil were added 15 g of sodium carbonate (Na_2CO_3), 8 mL of Sodium Hydroxide (NaOH) 0.1N and 3 mL of NaOH 1N.

2.3. Total metals

Previous to the analysis, soil samples of each soil were drying at 60 °C during 24 h. For determination of total metals in soil, samples were digested in a microwave MARSx CEM under conditions stated in the SW 846-3051 method [14].

The soil concentrations of As, Cd, and Pb were compared to the reference concentrations (Rc) in NOM 147, stated for residential and industrial use. The concentrations of Zn were compared with the reference level of ecological and human health risk indicated in the Federal Attorney for Environmental Protection of Mexico [15].

Peer-reviewed version available at Sustainability 2017, 9, 81; doi:10.3390/su9010081

2.4. Soluble metals

The extraction and analysis of soluble metals in each soil sample were based on the procedures in NOM-147, where the soil reacts with water liberating PTE.

An extracting agent solution (H_2O-CO_2 at pH= 5.5 ± 0.2) was prepared, 500 mL of the extracting agent solution were added to 25 g of soil and were agitated during 18 ± 0.25 h on a constant rotation equipment (environmental express). After agitation, the mixtures were filtrated and digested according to SW 846.3015A method [16]. The analysis of total and soluble metals (As, Cd, Pb, Zn) was done in Optic Plasma Equipment ICP Thermo Jarrel Ash IRIS/APDIV and in Atomic Absorption Spectrophotometer with Generation Hydride GBC AVANTA SIGMA.

2.5. Seed Germination

The experiment germination of the two grass species, *Bouteloua dactyloides* and *Cynodon dactylon*, was prepared in the collected soils A, B, C, D, E and a reference target F. Four repetitions with 30 seeds each were prepared by type of soil and grass specie. The petri dishes were irrigated every three days with the type of water corresponding to the pH of each soil (Table 1). The A soil was irrigated with distilled water (pH 5.4); B, C and F soils with faucet water (pH 7.9). For D soil, were prepared 2L of distilled water and added 0.5 mL of HCl 1N, to obtain acid water conditions (pH of 4.6). For E soil, 2L of distilled water were prepared adding 5.0 mL of NaOH 0.1N and alkaline conditions were obtained (pH of 9.0).

Table 1. Germination conditions for each type of soil.

Classification	pH Conditions for Germination	
	Soil pH	Irrigation Water pH
A	6.0	5.4
B	7.8	7.9
C	7.0	7.9
D	5.0	4.6
E	9.0	9.0
F	8.0	7.9

The prepared boxes were placed in a drying stove at a temperature of 28 °C ± 2 °C during germination time. Each third day, the germinated seeds of each box were counted, so the germination percentage and variation coefficient were compared by grass specie.

A Bartlett's test of homogeneity variance and an analysis ANOVA were done using Minitab 16, considering a 95% confidence for both grasses regarding the type of soil; the means, standard deviation, and variation coefficient (Vc) were compared. Also a general linear model (PROC GLM) in the SAS 9.1.3 software was considered, to compare germination between grasses, and a test of least significant difference (LSD) was done to compare germination between soils and grasses.

3. Results

3.1. Total metals

The metal concentration (As, Cd, Pb) of the collected soil exceeded the Rc for residential use in mexican regulation NOM-147 of 22 mg of As kg^{-1}, 400 mg of Pb kg^{-1} and 37 mg of Cd kg^{-1}. Furthermore exceeded the Rc of 260 mg of As kg^{-1} and 800 mg of Pb kg^{-1}, for soil of industrial use (Table 2).

The amount of Zn on the analyzed soil exceeded the Rc established in Mexico for ecological risk of 300 mg of Zn kg^{-1} and human health risk of 800 mg of Zn kg^{-1} [15] (Table 2).

Table 2. Total metal in soil.

		Concentration (mg kg-1)			
Soil	**pH**	**As**	**Cd**	**Pb**	**Zn**
A	6.0	2153.30	82.58	6340.38	8082.77
B	7.8	2447.15	98.72	6227.56	11441.56
C	7.0	1171.16	200.52	9172.16	9506.29
D	5.0	2956.18	126.89	5455.38	14502.99
E	9.0	2549.33	142.91	5516.44	15309.95
F	8.0	27.61	4.60	401.56	229.49

3.2. Soluble Metals

In Table 3 are shown the corresponding results for soluble-metals concentrations in mg L^{-1}. As varied from 0 0.07 to 0.13, Cd from 0.004 to 0.36, Pb from 0.02 to 0.10, and Zn 0.05 to 29.51. Only Cd exceeded the Rc of 0.10 mg L^{-1} indicated in NOM-147 (Soil D). The toxicity of PTE is proportional to the solubility of the solid stage in which they are associated [17].

Table 3. Soluble metal in soil.

		Concentration (mg L-1)			
Soil	**pH**	**As**	**Cd**	**Pb**	**Zn**
A	6.0	0.09	0.04	0.03	1.28
B	7.8	0.10	0.05	0.06	1.62
C	7.0	0.08	0.10	0.05	0.17
D	5.0	0.07	0.36	0.02	29.51
E	9.0	0.10	0.007	0.10	0.51
F	8.0	0.13	0.004	0.04	0.05

3.3. Seeds Germination

The germination time for both seeds was from 3 to 21 days. In Figures 1 and 2 is shown the behavior during 21 days of seed germination of B.dactyloides and C. dactylon by type of soil. B. dactyloides presented greater germination percentage from the fifth day in A, B, C, and F soils.

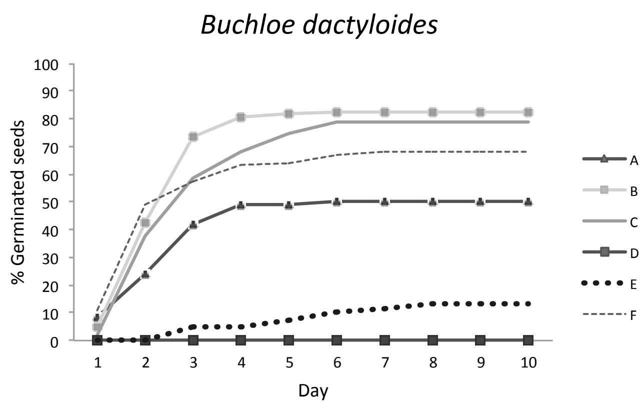

Figure 1. Germination percentage of B. dactyloides.

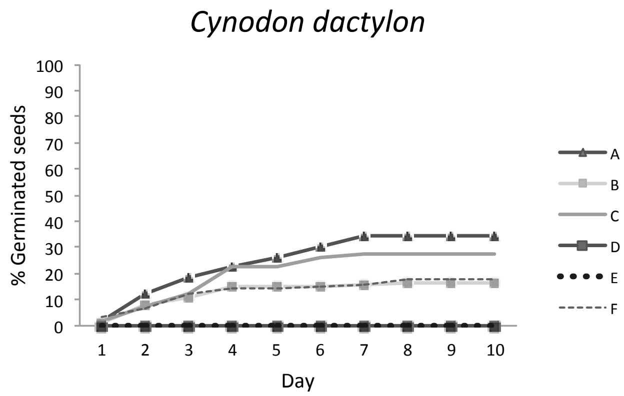

Figure 2. Germination percentage of C. dactylon.

The grass B. dactyloides presented a germination percentage from 68 to 83 % in soils with pH between 7 and 8 (B, C and F). A pH 6 (A) declined germination to 50%. An increased pH to 9 (E) caused a germination reduction to 13%, and an acid pH of 5 (D) did not germinate (Figure 1).

The germination percentage of C. dactylon was from 28 to 34 % in soils with pH of 6 and 7 (A and C). At pH > 7 (B and F) a germination reduction to 17 and 18 % was presented. With a pH of 5.0 (D) and 9.0 (E), germination was not reached (Figure 2).

According to Bartlett's test data exhibit variance homogeneity ($p>0.296$). PROC GLM model demonstrated that B. dactyloides and C. dactylon germination were significantly different ($R^2=0.9315$, $p<0.05$).

In LSD test ($p<0.05$) the least significant difference was 3.9473. For B. dactyloides demonstrated that germination in B and C soils was not significantly different (pH of 7.8 and 7.0, respectively), also C and F were not significantly different (pH of 7.0 and pH of 8.0, respectively) (Table 4), so C soil had similar germination development than the uncontaminated soil. A soil was not similar to any other germination soils; also B, C, and F soils had a higher germination mean than A.

For C. dactylon germination in A and C soils (pH 6 and 7, respectively) was not significantly different (soils with higher germination for this specie). Also, B and F soils (pH >7) were not significantly different, so B soil had similar germination development than uncontaminated soil (Table 4).

Table 4. Groups by LSD test

Group		Mean	N	Treatment
	a	24.75	4	BDB
	a			
b	a	23.75	4	BDC
b				
b		20.50	4	BDF
	c	15.00	4	BDA
	d	10.25	4	CDA
	d			
e	d	8.25	4	CDC
e				
e	f	5.25	4	CDF
e	f			
e	f	5.00	4	CDB

 doi:10.20944/preprints201612.0014.v1

Peer-reviewed version available at Sustainability 2017, 9, 81; doi:10.3390/su9010081

f			
f	4.00	4	BDE
g	0.00	4	CDD
g			
g	0.00	4	CDE
g			
g	0.00	4	BDO

In Tables 5 and 6 are shown germination percentage, mean of germinated seeds and variation coefficient (Vc) of each grass specie and soil type.

Table 5. Germination analysis of *B. dactyloides* in each soil.

Soil	pH	% Germination	Germinated seeds mean	Variation Coefficient (V_c)
A	6.0	50.0	15.0 ± 5.9	39.6
B	7.8	83	24.8 ± 3.4	13.8
C	7.0	79	23.8 ± 2.5	10.5
D	5.0	0	0	-
E	9.0	13	4.0 ± 3.6	89.0
F	8.0	68	20.5 ± 2.6	12.9

Table 6. Germination analysis of *C. dactylon* in each soil.

Soil	pH	% Germination	Germinated seeds mean	Variation Coefficient (V_c)
A	6.0	34	10.3 ± 3.0	29.1
B	7.8	17	5.0 ± 1.8	36.5
C	7.0	28	8.3 ± 1.0	11.6
D	5.0	0	0	-
E	9.0	0	0	-
F	8.0	18	5.3 ± 2.2	42.2

As far as the results of Vc the greater homogeneity presented on the grass germination was on B. dactyloides in soils B and C with a Vc of 13.8 and 10.5%, respectively. Meanwhile, on C. dactylon was C soil with an 11.6% (Tables 4 and 5).

Grass germination reached a peak and stabilized, this was determinate with a regression of orthogonal contrast, so the model proved being square ($P<0.0001$).

3.4. Discussion

B. dactyloides showed a better germination percentage than *C. dactylon* on soils A, B, C, E and F. This can be associated to that different plant species own distinctive seed's coat anatomy and structure; so the same metal concentration can have dissimilar effect in different species [10]. Therefore *C. dactylon* showed being less tolerant to As, Cd, Pb and Zn pollution. Also, it can be supposed that this grass specie needs different germination conditions, because even the control soil had a low germination percentage.

In both grasses there was no germination in D soil. Although, D soil did not presented higher concentration of total metal than the other soils, it was the soil with lower pH and also higher

Peer-reviewed version available at Sustainability 2017, 9, 81; doi:10.3390/su9010081

bioavailability of soluble Cd and Zn, in fact Cd higher than the Rc (Table 3). This metal solubility could cause the lack of development of both species, due soil pH has a high influence in the bioavailability of metals and metalloids. Acid soil pH makes metals more soluble and, therefore, their bioavailability is greater for plants [18].

Cd has been defined to interfere with the water uptake and therefore the germination does not occur [10]. Cd has also been reported to cause chromosomal aberrations, with cytogenetic analysis of Pisum sativum root from seeds germinated on different concentrations of Cd was demonstrated the increased frequency of abnormalities. The total number of aberrations increased correlated with Cd concentration [8].

On other hand, Zn is one of the essential micronutrients in plants, it is necessary for normal plant growth and development, and also is required in several metabolic processes. Nevertheless, at high concentration is toxic and hampers plant growth [3]. The excess of Zn affects the uptake of other nutrients, inhibiting seed germination, plant growth, and root development [10].

In rapeseed exposed to 1.12 mM Zn, was determinate a plant growth inhibition and chlorosis in leaves. The contents of Fe, Cu, Mg and Mn decreased in roots and leaves. Also, was demonstrated that Mg and Cu contents decreased with increased Zn concentration in ryegrass leaves. These results indicated that excess Zn might suppress the uptake of these elements, due to the competition among metals [19]. Therefore, a research with sunflower determinate that seeds germinated at high Zn concentrations while germination decreased to below 50% after exposure to Cd, Cu and Pb [8].

Marichali et al., (2014) achieved germination of C. sativum seedling irrigated with high Zn concentration at a pH of 6.8, but reduced root length. They determinate that for C. sativum, Zn has low toxic effects on germination, and roots are more sensitive than seeds to metal stress [3]. This result does not agree with the present research possibly because the pH and the germination conditions were different.

The bioavailability of Cd, Pb, and Zn decreases while soil pH increases due to its precipitation as insoluble hydroxides, carbonates and organic complexes, and opposite As bioavailability increases at basic pH [20]. This can explain why B. dactyloides reduced germination in E soil (Table 4) with similar metal contamination level than the other soils (Table 2), but with a basic pH that induce more As bioavailability and with higher As solubility (Table 3). C. dactylon exhibited reduced germination above neutral pH (Table 5), indicating that this specie is sensitive to As presence. The most common genotoxic effects reported for As exposure involve mitotic spindle disturbance. In Hordeum vulgare seeds the frequency of chromosomal abnormalities was proportional to the concentration of As [8].

C. dactylon also showed less tolerance to Cd and Zn possibly due to the effects discussed above. Also, could be affected by Pb, due an excess of this metal in some plants inhibits seed germination [2]. Hence, it is not effective for soil phytoremediation contaminated by As, Cd, Pb and Zn, because metal tolerance is an important factor for the use of plants and seeds for this technology [8].

For soil remediation, the capacity of seeds to germinate in the presence of metals and metalloids could be a primary concern [8]. In this research was confirmed that, metals generally cause inhibition of germination and seedling growth [21], but the metal distribution in seeds and affectation degree differs depending on the metal involved, plant specie and seed anatomy. Thus, seeds of metal tolerant plants may have a higher threshold for toxicity than non-tolerant ones [8]. After permeation through the seed coat, the germination relies on the seed reserves for metabolites supply for respiration, metals can cause stress and disrupt the process, and interfere with the enzymes involved in the germination [10].

4. Conclusions

The concentration of total and soluble metals in the used soils for germination, confirmed that the site were the soil was collected is highly polluted with As, Cd, Pb and Zn.

The germination of Bouteloua dactyloides was greater than Cynodon dactylon, on polluted soils with As, Cd, Pb, and Zn.

In soil at pH 5, the germination of both species was not reached, owing that metals are more bioavailable in acid environments; moreover Cd and Zn in this soil were more soluble than in the others. *C. dactylon* do not even germinated at basic conditions in soil (pH 9) were As is more bioavailable.

C. dactylon is less tolerant to As, Cd, Pb and Zn, so is considered not effective for a phytoremediation process. *B. dactyloides* tolerate the metal contained in this soils, this grass grow at 20 cm maximum, which gives an opportunity of covering the surface of the tailings dams with a vegetal cover. Nevertheless, in future research with this specie is necessary to evaluate the soil pH and soluble metal before begin the remediation process. In addition, more studies are needed to investigate the effects of metals on seed germination and plant growth in situ.

Acknowledgments: To the Autonomous University of Chihuahua and the Advanced Materials Research Center for the economical support.

Author Contributions: M. Rosario Delgado-Caballero performed the experiment and all the analysis conducted. M. Teresa Alarcón-Herrera headed the experiment performance and the data analysis. M. Cecilia Valles-Aragón analyzed data and discusses the results obtained, she organized the structure of this paper. Melgoza-Castillo was involved in the experimental performance. Leyva-Chávez collaborated with the statistic analysis of the data. Ojeda-Barrios take part on this project because her expertise on plants physiology, so she assisted on define the development of both grasses.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. USEPA. Residential lead hazard standards-TSCA section 403: office of pollution prevention and toxics; United States Environmental Protection Agency: USA, 2001.
2. Kumar, R.; Kumar Mishra, R.; Mishra, V.; Qidwai, A.; Pandey, A.; Kant Shukla, S.; Pandey, M.; Pathak, A.; Dikshit, A. Detoxification and tolerance of heavy metals in plants. Plant Metal Interaction 2016, Chapter 13, DOI: 10.1016/B978-0-12-803158-2.00013-8. Available online: https://www.researchgate.net/publication/301242340_Detoxification_and_Tolerance_of_Heavy_Metals_in_Plants (accessed on 30 July 2016).
3. Marichali, A.; Dallali, S.; Ouerghemmi, S.; Sebei, H.; Hosni, K. Germination, morpho-physiological and biochemical responses ofcoriander (Coriandrum sativum L.) to zinc excess. Ind Crop Prod 2014, 55, 248-257. DOI:10.1016/j.indcrop.2014.02.033. Available online: http://www.sciencedirect.com/science/article/pii/S0926669014001113 (accessed on 30 July 2016).
4. Chul Kong, I. Joint effects of heavy metal binary mixtures on seed germination, root and shoot growth, bacterial bioluminescence, and gene mutation. J Environ Sci 2013, 25, 889-894, DOI: 10.1016/S1001-0742(12)60174-0. Available online: http://www.sciencedirect.com/science/article/pii/S1001074212601740 (accessed on 15 May 2016).
5. Gutiérrez, M.E.; Moreno, M. Los residuos en la minería Mexicana; Instituto Nacional de Ecología (INE): D.F., México, 1997.
6. SEMARNAT. Información proporcionada por la Dirección General de Gestión Integral de Materiales y Actividades Riesgosas; Secretaría de Medio Ambiente y Recursos Naturales. México, 2004.
7. Gulson, B.L.; Masón, K.J.; Korsch, M.J.; Howarth, D. Non-orebody sources are significant contributors to blood lead of some children with low to moderate lead exposure in a mayor mining community. Sci Total Environ 1996, 181, 223-230. DOI:10.1016/0048-9697(95)05015-9. Available online: http://www.sciencedirect.com/science/article/pii/0048969795050159 (accessed on 25 November 2015).
8. Kranner, I.; Colville, L. Metals and seeds: Biochemical and molecular implications and their significance for seed germination. Environ Exp Bot 2011, 72, 93-105. DOI:10.1016/j.envexpbot.2010.05.005. Available online: http://www.sciencedirect.com/science/article/pii/S0098847210001164 (accessed on 25 November 2015).
9. Salt, D.E.; Smith, R.D.; Raskin, I. Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 1998, 49, 643-668. DOI: 10.1146/annurev.arplant.49.1.643. Available online: http://www.annualreviews.org/doi/abs/10.1146/annurev.arplant.49.1.643 (accessed on 25 July 2016).

10. Márquez-García, B.; Márquez, C.; Sanjosé, I.; Nieva, F.J.J.; Rodríguez-Rubio, P.; Muñoz-Rodríguez, A.F. The effects of heavy metals on germination and seedling characteristics in two halophyte species in Mediterranean marshes. Mar Pollut Bull 2013, 70, 119-124. DOI:10.1016/j.marpolbul.2013.02.019. Available online: https://www.ncbi.nlm.nih.gov/pubmed/23465623 (accessed on 25 July 2016).
11. Brede, D. Turfgrass maintenance reduction handbook: sports, lawns, and golf; Sleeping Bear Press: Chelsea, MI USA, 2000; pp. 386, ISBN 1-57504-106-5.
12. USDA NRCS. Plant Materials Program; United States Department of Agriculture Nature Resources Conservation Service, 2000. Available online: http://npdc.usda.gov (accessed on 21 November 2015).
13. NOM-147-SEMARNAT/SSA1-2004. Que establece Criterios para determinar las Concentraciones de remediación de suelos contaminados por arsénico, bario, berilio, cadmio, cromo hexavalente, mercurio, níquel, plata, plomo, selenio, talio y/o vanadio; Secretaría de Medio Ambiente y Recursos Naturales: D.F., México, 2004.
14. USEPA. Method 3051 Microwave Assisted Acid Digestion of Sediments, Sludges, Soils and Oils; United States Environmental Protection Agency: USA, 2007a. Available online: https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf (accessed on 21 November 2015).
15. PROFEPA. Disposición y procedimientos para la caracterización y restauración de suelos contaminados; Secretaría de Medio Ambiente y Recursos Naturales, Procuraduría Federal de Protección al Ambiente, Subprocuraduría de Auditoría Ambiental, Dirección General de Emergencias Ambientales: D.F., México, 2000; pp. 225.
16. USEPA. Method 3015A microwave assisted acid digestion of Aqueous samples and extracts; United States Environmental Protection Agency: USA, 2007b. Available online: https://www.epa.gov/sites/production/files/2015-12/documents/3015a.pdf (accessed on 21 November 2015).
17. Traina, S.; Laparche, V. Contaminant bioavailability in soils, sediments and aquatic environments. In Colloquium "Geology, Mineralogy, and Human Welfare", Irvine, California, November 8-9; Proceedings of the National Academy of Sciences of the United States of America, 1999; 96, 3365-3371.
18. Ross, S.M. Toxic metals in soil-plant systems, John Wiley & Sons: New York, USA, 1994; pp. 63-152. ISBN: 978-0-471-94279-5.
19. Wang, D.; Zhang, S.H.; Wang, P.F.; Hou, J.; Zhang, W.J.; Li, W.; Lin, Z.P. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. Chemosphere 2009, 75, 1468–1476, DOI: 10.1016/j.chemosphere.2009.02.033. Available online: http://www.sciencedirect.com/science/article/pii/S0045653509002203 (accessed on 18 June 2016).
20. Silveira, M.L.A.; Alleonl, L.R.F.; Guilherme, L.R.G. Biosolids and heavy metals in soils. Sci AgriC 2003, 60, 793-806, DOI: 10.1590/S0103-90162003000400029. Available online: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-90162003000400029 (accessed on 15 November 2015).
21. Bae, J.; Benoit, D.L.; Watson, A.K. Effect of heavy metals on seed germination and seedling growth of common ragweed and roadside ground cover legumes. Environ Pollut 2016, 213, 112-118, DOI: 10.1016/j.envpol.2015.11.041. Available online: http://www.sciencedirect.com/science/article/pii/S0269749115302013 (accessed on 30 July 2016).