Assessment of Potential Ecological Adaptation of the Species *Miscanthus Giganteus* in Conditions of Fly Ash Deposit

Benoni Lixandru*, Florica Morariu, Dumitru Popescu

Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Faculty of Animal Science and Biotechnologies, 300645-Timisoara, Calea Aradului 119, Timis, Romania

Abstract
Miscanthus giganteus is a large, perennial (up to 20 years) grass hybrid of *M. sinensis* and *M. sacchariflorus* native to Japan. It is a sterile hybrid, therefore it propagates vegetative through its rhizomes and that it is a completely non-invasive species. For three years we have monitored a culture of *Miscanthus Giganteus* on an area of 300 m^2, divided into three experimental groups: fertilization with 50 $t*ha^{-1}$ sewage sludge; fertilization with 50 $t*ha^{-1}$ manure; 100 $kg*ha^{-1}$ $N_{15}P_{15}K_{15}$. Only in the first year of vegetation was assured irrigation. In the first year of vegetation was observed that the species *Miscanthus giganteus* can adapt well to the conditions of fly ash dumps biotope in the case of organic fertilization with sewage sludge and with cattle manure. In the second and third years was observed that again organic fertilization with sewage sludge and with cattle manure have produced the higher vegetative mass, but we observed that inorganic fertilized plot have a good evolution, even in a slow rate. In conclusion we can affirmed that a culture of *Miscanthus Giganteus* grown in conditions of fly ash deposit improve the environment condition in an ecological way.

Keywords: adaptation, fly ash, *Miscanthus giganteus*

1. Introduction

Miscanthus giganteus is a large herbaceous species that belongs to the family Andropogoneae (Figure 1).

Figure 1. *Miscanthus giganteus*

It originated in Yokohama, Japan, from where it was brought to Denmark by Aksel Olsen in 1935. From there it spread in Europe and then in North America. It is a perennial species and it is resistant to environmental conditions. It is often incorrectly called "elephant grass", a syntagma which actually belongs to the species *Pennisetum purpureum*. Over time it was known under different names such as *Miscanthus sinensis* 'Giganteus', *M. giganteus*, *Miscanthus ogiformis* Honda, and *Miscanthus sacchariflorus* var. *brevibarbis* (Honda) Adati [1].

Recently, following research from the Royal Botanic Gardens at Kew, England, is established that *Miscanthus giganteus* is a sterile hybrid resulting from the crossing of species *M. sinensis* and *M. sacchariflorus* [1-4]. In fact, it is an allotriploid with a number of chromosomes 3n=57 [5]. The longevity of this species can reach up to 20 years, and can grow to a height of 3-4 m. The energy output per hectare is up to 20 t SU. Due to its particular energy potential, *M. giganteus* was

* Corresponding author: Lixandru Benoni
Tel. +40256277142, E-mail: benlix@animalsci-tm.ro

classified as a C4 energy plant [6]. It multiplies through rhizomes (Figure 2).

Figure 2. Rhizomes of *M. giganteus*

Since 1983 it has been researched and studied in Europe to produce, by burning, thermal and electrical energy [7, 8]. Since 2005, the US government has encouraged research into green energy, using herbaceous plants as raw materials for producing ethanol as biofuel for transport. In this respect, due to its potential to produce large amounts of biomass, there was given great importance to the species *M. giganteus* [9].

In our country, the first company which imported it from Austria was ARGE Miscanthus (www.miscanthus.com.ro). Following experiments conducted in Copşa Mică, by Horia Barbu from the "Lucian Blaga" University of Sibiu and by Teodor Vintilă form USAMVB Timişoara, with rhizomes from the company ARGE, there have been obtained good results even on land unsuitable for cereal crops [10]. As a result of the accumulated experience, specialists form the company ARGE in Romania show that *M. giganteus* can be grown on land contaminated with sterile soil and fly ash, it can be used as a protective curtain around deposits of toxic waste and garbage, as protective curtain against snow blizzards on roads or even as an ornamental plant. According ISTIS data (State Institute for Varieties Testing) in Bucharest, the Art variety of this species was recorded in the national Catalogue of varieties of crop plants in Romania, from 18.XII.2012.

Knowing the remarkable biotic potential of this species, resulted from resistance, longevity and abundance of biomass, it was considered that *M. giganteus* could become an important factor of stabilization and revegetation of power plant ash in deposits.

Therefore, following an old collaboration between SC COLTERM S.A. Timişoara and USAMVB Timisoara, regarding the trials of stabilization and revegetation of power plant ash deposited in the location next to Utvin town, in Timis County and in accord to the company ARGE Miscanthus in Romania, it was decided the planting of *Miscanthus giganteus* rhizomes on fly ash in one of the existing sectors in the said location. During a period of three years, it was monitored the growth and development of this species to determine its ability to adapt to a barren biotope, devoid of any organic material (Figure 3).

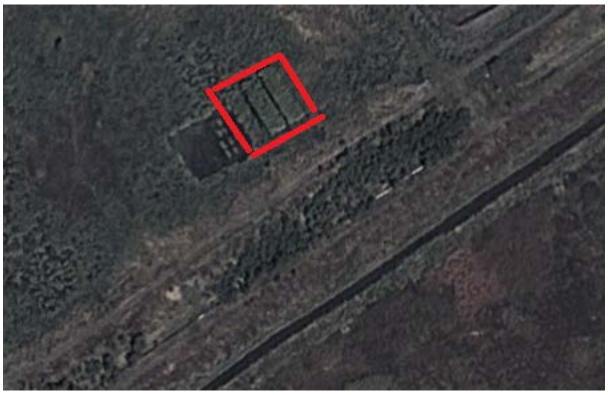


Figure 3. Location of the experiment on fly ash dump near Utvin, Timis County

2. Materials and methods

Since fly ash is a mineral residue devoid of organic matter, it was intended to supplement with organic and mineral fertilizers applied directly to the nest planting. For this, we used semi-wet sludge from municipal sewage treatment plant of Aquatim Timisoara, well fermented manure from the didactic resort USAMVB Timisoara and the $N_{15}P_{15}K_{15}$ fertilizer complex. Rhizomes of *M. giganteus* came from Austria, being brought by the company ARGE Miscanthus. Planting was carried out in 10 May 2012.

Experimental block consisted of three equal plots (variants), each with an area of 100 m^2 (20x5 m):

- Variant V1- sludge fertilization (dose: 50 t/ha and 5 kg/plant);
- Variant V2 - fertilization with manure (dose: 50 t/ha and 5 kg/plant);
- Variant V3 - mineral fertilization (dose: 100 kg $N_{15}P_{15}K_{15}$/ha and 10 g/plant).

Planting rhizomes was made on 5 rows, with 20 nests at a time. This resulted in 100 cores per plot, returning a 1 m^2 area of nutrition/plant and rhizome. Planting nests were dug to a depth of 15 cm. After mixing ash with fertilizer there was applied the rhizome, it was covered with ashes, and finally nest settlement was made (Figure 4).

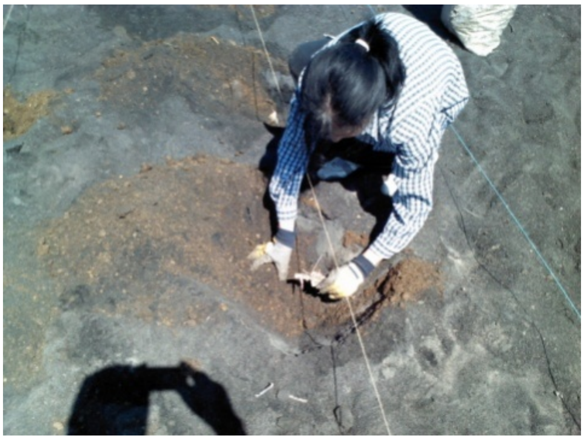


Figure 4. Planting of *M. giganteus* rhizomes

Between May and September of the first year, the experimental plots were watered. With the technical support of SC Colterm SA, there were mounted three sprinklers and there was established a watering system, three times a week, about 2 hours per watering.

During the first year, we analyzed the following biological parameters: the ability to sprout from planted rhizomes, number of tillers/nest, the maximum height of sprouts and biomass production at harvesting. Each year, the harvest was done in early March. In the second and third years, maximum height of sprouts was measured and biomass production was determined at the annual harvesting.

3. Results and discussion

One month after planting, it was found that there were nests were rhizomes had not formed sprouts. The number of pockets with dead rhizomes was 3 in V1, 8 in V2 and 9 in V3, resulting in a capacity of sprouting of 97% in V1, 92% in V2, and 91% in V3 (Table 1).

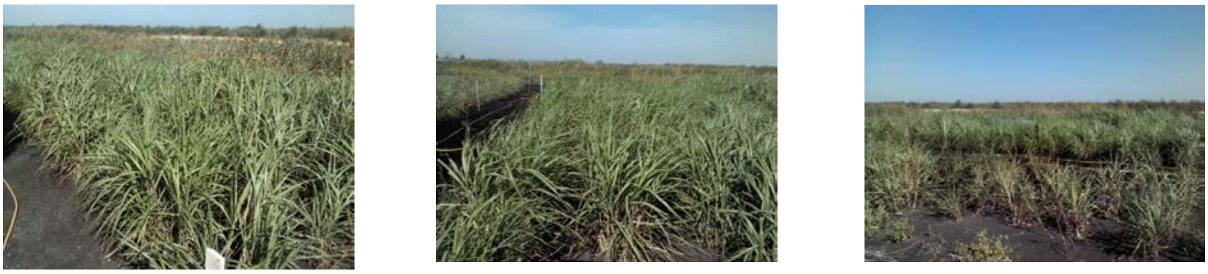
Table 1. Growth and developing indices of plants of *M. giganteus* species during the first three years of the establishment of crop

Specification	Experimental variants		
	V1 (sludge)	V2 (fermented manure)	V3 (NPK)
1.On first harvest (2013) [11]			
Nr. of rhizomes planted / nr. of rows	20/5	20/5	20/5
Sprouting capacity (%)	97	92	91
Average nr. of sprouts / nest ($\bar{x} \pm s_{\bar{x}}$)	27.52±1.26*)	26.76±2.02*)	8.32±3.22
Average height of sprouts (cm) ($\bar{x} \pm s_{\bar{x}}$)	165±2.23*)	123 ± 3.66*)	45 ± 2.01
Biomass production at harvest (t/ ha)	3.15	3.01	0.6
2. On second harvest (2014)			
Average nr. of sprouts/nest	32.4	33.9	18.6
Average height of sprouts (cm)	3.10	2.80	2.55
Biomass production at harvest (t / ha)	15	13.3	3.6
3. On third harvest (2015)			
Average nr. of sprouts/nest	51.2	52.6	42.7
Average height of sprouts (cm)	3.80	4.02	3.63
Biomass production at harvest (t / ha)	23	25	20

*) $p \leq 0.01$

After nearly three months of vegetation, plants have had a very good development for the variants organically fertilized with sludge and manure and a weaker development in the variant with chemical fertilizer. At this time, plant height ranged from 90-170 cm in V1, between 70 and 150 cm in V2 and from 30-120 cm in V3 (Figure 5). There can be observed the proper development of plants in variants V1 and V2.

In the first decade of March of the following year, were

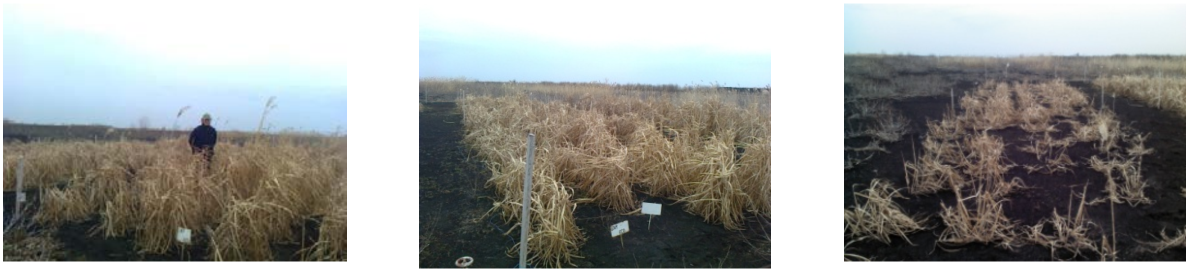


Variant V1 Variant V2 Variant V3

Figure 5. *Miscanthus giganteus* plants three months after planting

measured the biological parameters and was done the harvest of above-ground plant biomass production.

After the first growing season, it appears that *M. giganteus* plants that received organic fertilizer, recorded the best results. In variants V1 and V2, sprouts showed a luxuriant growth, showing an average of over 26-27 tillers/plant. In the non-organic fertilized variant (V3), the average number of sprouts was over 3 times lower ($p \leq 0.01$). Also, in the organic fertilized variants sprouts had a significantly greater height ($p \leq 0.01$) compared to chemically fertilized variant. Sprout height, density and uniformity of the plants are features prominently shown in organic fertilized plots. Consequently, there resulted a uniform and compact aspect of vegetation in the two variants compared to variant V3 (Figure 6).



Variant V1 Variant V2 Variant V3

Figure 6. Culture of *Miscanthus giganteus* after the first year of vegetation (March 2013)

Dry biomass production after the first year of vegetation was almost 6 times higher in organically fertilized variants (3145 kg/ha in variant V1 and 3010 kg/ha in variant V2) versus mineral fertilized variant.

We emphasize that the production of biomass from species *M. giganteus* has, during its longevity, a continuous growth and development, reaching the full potential after 3-4 years. Moreover, due to the lack of profitability, in the first year is not recommended harvesting biomass for biofuel.

In the second year of culture, due to biodegradation of the dead leaves fallen on the ground after the end of the growing season (Figure 10), in the area of germination of rhizomes from the planting nests there has accumulated a certain amount of organic material that has stimulated the process of sprouting and then a more vigorous plant development during vegetation. This aspect is well emphasized by biomass obtained from the second harvest, which recorded rates of 15 t/ha in V1, 13.3 t/ha in V2 and 3.6 t/ha in V3. These results show an increase of over 5 times of biomass production of the first year. During the second year, the *M. giganteus* plants had a very good development, resulting in a compact soil cover at the base of the plants which favored the preservation of moisture at the surface of the ash stratum (Figures 7 and 8).

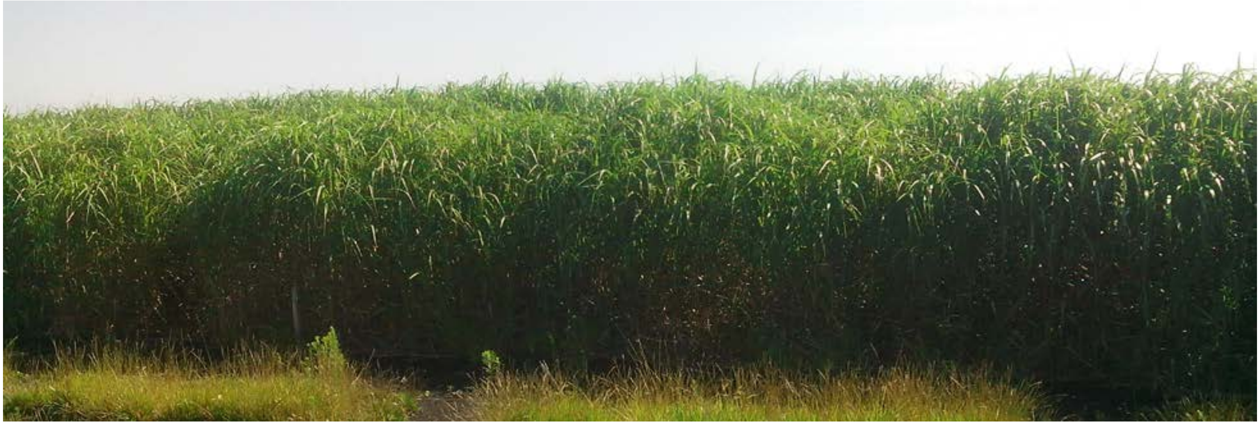


Figure 7. Culture of *Miscanthus giganteus* in the second year of vegetation

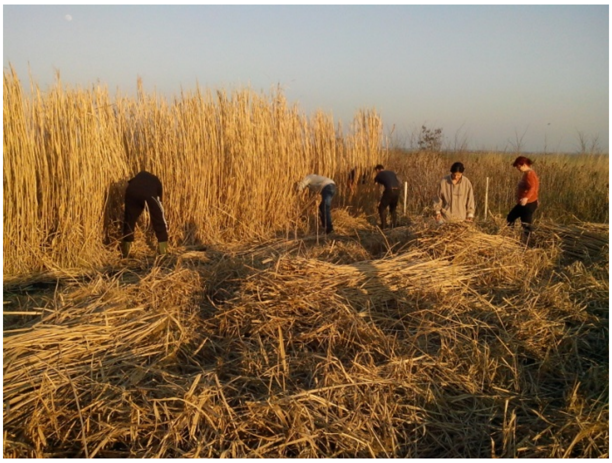


Figure 8. Harvest of crop biomass production in the second year of vegetation

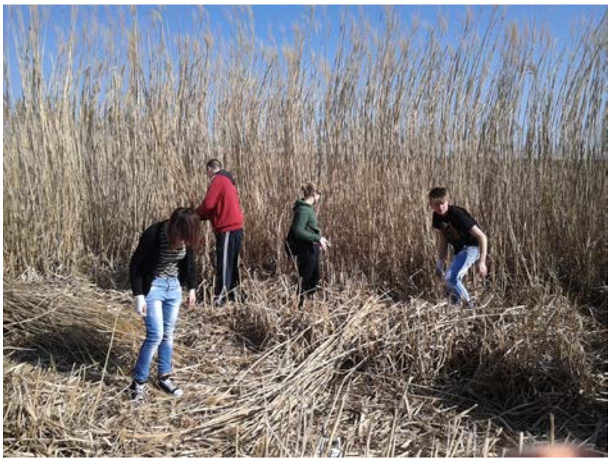


Figure 9. Harvest of crop biomass production in the third year of vegetation

The results obtained in the third year of culture confirm all expectations. Throughout the growing season, plants had a very good development. There has been continued the sprouting and foliar development horizontally, which led to increasing the degree of soil coverage and shading. There resulted a thickening and a horizontal compaction of phytocoenosis, coupled with a very good height growth rate of foliar floors, which favored the cancellation of the aridity index from the surface of the layer of ash and maintaining moisture at the base of the plants (Figure 10). Compared to the first year, the average number of tillers/nest increased with 86% in V1, 97% in V2 and over 5 times in V3. In the third year of harvesting, plant height was 3.6 to 4.0 m and biomass production was 23 t/ha in V1, 25 t/ha in V2 and 20 t/ha in V3 (Figure 9).

It is noted that in all three variants, including the *Miscanthus* variant non-organically fertilized, there was obtained production exceeding 20 t/ha, comparable to those of similar experiments (Acaroglu and Aksoy, 1998 [9]; Anderson et al., 2011 [9]).

For our experiment, the vegetation cycle for the species *Miscanthus giganteus* was completed during September-October months when the leaves turn yellow, some of which fall, forming a thick layer on the surface, subsequently enriched after harvesting activities (Figure 10). This results in a thermal protection of the rhizomes during winter, and after their biodegradation, in soil enrichment with organic material.

Due to these biological processes, we believe that in our experiment there were noted good results for two reasons. The first one is represented by the high values, of 23-25 t/ha biomass harvested after just three years, close to the biological maximum of the species. Secondly, we emphasize that this biomass production was obtained on arid habitat of a fly ash dump site, free of organic material.

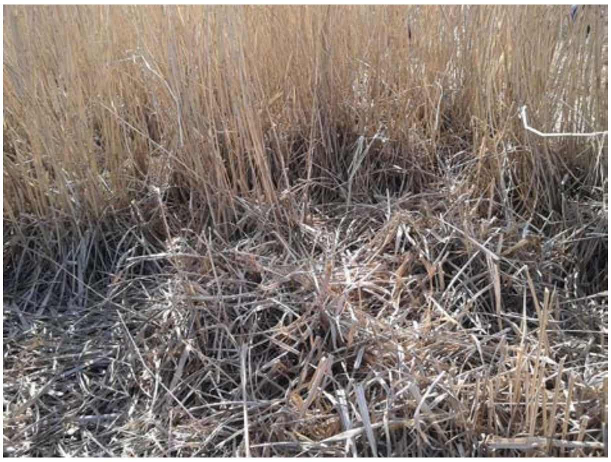


Figure 10. The layer of biodegradable phytomass remaining on the soil surface after harvest

However, *Miscanthus giganteus* species has adapted and developed very well, especially after the second and third year after the planting of rhizomes. It is true that in the first year after planting, in the June-August period, the plants were irrigated 2-3 times a week in a watering regime, for several hours a day. Also, given the mineral composition of ashes at planting, it is recommended to ensure an organic supplement of about 5 kg of fermented manure or sewage sludge per nest. Use of sewage sludge, resulting in large amounts at wastewater treatment plants, can be a method of sustainable use of the organic waste in the revegetation work of completed sectors of the fly ash deposit.

4. Conclusions

Establishing *Miscanthus giganteus* crops on the fly ash wastelands, resulting from the burning of lignite, can be a feasible agro-technical solution, by which, with minimum costs, is obtained the revegetation of this arid biotope and the blocking of ash deflation.

Since the ashes of the warehouse do not contain organic material, organic fertilization of rhizomes planting nests with a 5 kg/plant is necessary. For this, one can use sludge or fermented manure.

Irrigation in the first year of vegetation, three times a week, is a basic agro-technical requirement for with high temperatures and dry periods in summer.

Given the ecological use of organic waste resulting from wastewater treatment in urban areas, we recommend as organic fertilizer the sewage sludge applied in the nest at planting rhizomes.

Three years after planting, harvested biomass production is 20, 23 and 25 t/ha which proves a successful adaptation, distinguished by simple agro-technical works, such as organic fertilization and irrigation during the summer months in the first year of vegetation.

References

1. Hodkinson, T. R., Renvoize, S., Nomenclature of Miscanthus × giganteus (Poaceae), Kew Bull, 2001, 56, 759-760

2. Scally, L., Hodkinson, T. R., Jones, M. B., Origins and taxonomy of Miscanthus. In: Miscanthus for energy and fibre. Jones, M. B., Walsh, M., Ed. James & James, London, UK, 2001, pp. 1–9

3. Hodkinson, T. R., Chase, M. W., Takahashi, C., Leitch, I. J., Bennett, M. D., Renvoize, S. A., The use of DNA sequencing (ITS and TRNL-F), AFLP, and fluorescent in situ hybridization to study allopolyploid Miscanthus (Poaceae)., Am. J. Bot., 2002, 89, 279–286

4. Hodkinson, T. R., Chase, M.W., Renvoize, S. A., Characterization of a genetic resource collection for Miscanthus (Saccharinae, Andropogoneae, Poaceae) using AFLP and *ISSR PCR.*, Ann. Bot., 2002, 89, 627–636

5. Yu, C. Y., Hyoung, S. K., Rayburn, A. L., Widholm, J. M., Juvik, J. A., Chromosome doubling of the bioenergy crop, Miscanthus × giganteus. Glob. Change Biol. Bioenergy, 2009, 1(6), 404–412

6. Acaroglu, M., Aksoy, A. S., Third year growing results of C_4 energy plant Miscanthus sinensis in producing energy from biomass. In: Biomass for Energy and Industry, Proc. 10th EU Bioenergy Conference, Wurzburg, Germany, June 1998. C.A.R.M.E.N. Publishers, Rimpar, Germany. 1998, pp. 758-759

7. Venturi, P., Huisman, W., State of art of the production of Miscanthus x giganteus in Europe. In: Making a Business from Biomass, Proc. 3rd Biomass Conference of the Americas, Montreal. Ed. Overend, R.P., Chornet, E., Pergamon/Elsevier Publishers, 1997, pp. 155-165

8. Lewandowski, I., Clifton-Brown, J., Scurlock, J. M. O., Huisman, W., Miscanthus: European experience with a novel energy crop. Biomass Bioenergy, 2000, 19, 209–227

9. Anderson, E., Rebecca Arundale, Maughan, M., Oladeinde, A., Wycislo, A., Voigt, T., Growth and agronomy of Miscanthus × giganteus for biomass production. Biofuels, 2011, 2(2), 176-183

10. Vintilă, T., Monica Dragomirescu, Veronica Croitoru, Cornelia Vintilă, Barbu, H., Camelia Sand, Saccharification of lignocellulose - with reference to

Miscanthus-using different cellulases. Romanian Biotechnological Letters, 2010, 15(4), 5498-5504
11. Lixandru, B., Dragomir, N., Morariu, F., Popa, M., Coman, A., Savescu, N., Masu, S., Popescu, D., Researches Regarding the Adaptation Process of the Species *Miscanthus Giganteus* under the Conditions of Fly Ash Deposit from Utvin, Timis County. Scientific Papers: Animal Science and Biotechnologies, 2013, 46 (1), 199-203