

STATISTICAL ANALYSIS OF THE FACTORS THAT INFLUENCE THE PHYTOREMEDIATION OF A CONTROLLED HAPLIC LUVISOIL POLLUTED WITH CRUDE OIL IN ORADEA, BIHOR COUNTY

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Abstract

Among the technologies used for the bioremediation of agricultural land polluted with petroleum residues, phytoremediation is considered the most environmentally-friendly and most economically-efficient due to reduced costs.

The objective of this paper is the statistical analysis of the variables that affect the yield differences (YD) made during the phytoremediation of a controlled polluted soil, with 3 % crude oil, obtained in 10 years of experimental observations in the field from the Agricultural Research and Development Station Oradea.

The yield differences made by the polluted variant in relation to the unpolluted variant provide information about the concentration of crude oil remaining in the soil at some moment of the phytoremediation process. These are correlated with the time, T (years) and the mean temperature from March – August, MT III-VIII (°C).

The second-order polynomial correlation with two factors and their interaction, very significant statistically, between YD as a dependent variable and the independent variables T and MT III-VIII allow the establishment of very important characteristics of the phytoremediation process. The duration of the ecological reconstruction differs from 8.5 - 9.5 years for MT III-VIII of 16 °C to 4-5 years for MT III-VIII of 18 °C.

Key words: soil pollution, phytoremediation, yield differences, statistical analysis

INTRODUCTION

Ecological restoration or remediation of contaminated or polluted soils with petroleum residue is a very complex activity due to the very diverse soil characteristics, the complexity of pollutants and the multitude of applicable technologies. Bioremediation, which consists in the biodegradation of pollutants from different media, air, water or soil, through biological processes specific to the living world, plants, animals, microorganisms for mitigation, transformation or elimination of toxic substances, detaches itself from the multitude of other technologies (Atlas, 1981; Elsner et al., 2004; Wang et al., 2012).

In the case of agricultural lands polluted with crude oil or petroleum residues, when these are cantoned on the superior part of the soil profile, usually “on-situ” bioremediation technologies are applied, out of which

phytoremediation is considered among the most environmentally-friendly and economically efficient technologies from the costs viewpoint (Banks, Schultz, 2005; Glick, 2010; Ichim et al., 2010). Phytoremediation is part of bioremediation technologies with the help of green plants, which by accumulating different types of bacteria in the area of the rhizosphere, degrades, adsorbs, translocates, accumulates and stabilizes the pollutants from soil (Nie et al., 2011; Muratova et al., 2012; Gkorezis et al., 2016).

Large scale application of bioremediation technologies of polluted soils with petroleum products is only possible after field or laboratory studies on the characteristics of the pollutant, soil and factors that influence the ecological reconstruction process, tolerant crop structure, methods for process intensification, amendments, fertilizers, soil works, climatic conditions, etc. (Urs, Micle, 2010; Răducu et al., 2012; Sabău, Șandor, 2013).

In order to study the bioremediation possibilities of polluted soils from Suplacu de Barcău, between 1993 and 2003, at the Agricultural Research and Development Station Oradea, two experiments were carried out on the effect of crude oil-controlled pollution on agricultural products (phytoremediation) and the effect of the application of an organic and mineral fertilization system upon the intensification of the bioremediation process of polluted soil by 3 % of crude oil was studied (Colibaș et al., 1995; Șandor, 2011; Șandor et al., 2013).

The obtained data during the studies are statistically analyzed, using specific methods of calculation, in order to establish the type of relationships existing between the factors that influence the intensity of the biodegradation process and the determination of their statistical significance (Săulescu, Săulescu, 1967; Ardelean et al., 2005; Sabău, Șandor, 2015).

Taking in consideration that the experiences from Oradea were cultivated with two cultures considered tolerant to oil pollution, millet in the first three years and spring wheat in the last seven years, the percentage yield difference (YD) between the 3 % polluted variants and unpolluted, control variants were used for the monitoring of crude oil biodegradation (Sabău, Șandor, 2014).

The objective of the present paper is the statistical analysis of the variable mean yield differences YD (%) and the factors that influence the evolution of the pollutant concentration during the phytoremediation process. The independent variables are the crops, time T (years), precipitation sum from March to August PS III-VIII (mm), mean temperatures during the same period MT III-VIII (°C) and de Martonne aridity index values (dMAI III-VIII).

MATERIAL AND METHOD

Due to the fact that most soils polluted with crude oil in Romania are located on luvisols, the experiences regarding the phytoremediation of the polluted soil with crude oil and its intensification by applying the organic and mineral fertilization systems at the Agricultural Research and Development Station from Oradea were located on a haplic luvisol (Colibaş et al., 1995).

The first experience is placed under the form of a Latin square, with parcels of 1 m², polluted with 0, 1, 3, 5 and 10 % of the crude oil brought from Suplacu de Barcau, Bihor County, in 4 repetitions. The experience of intensifying the phytoremediation process is tri-factorial, of the 2 x 4 x 4 type, with subdivided plots, placed randomly in 4 repetitions, the micro particles having 1 m². The studied factors were: A - controlled pollution: a₀ - unpolluted, control, a₁ - polluted with 3 % crude oil on the plowed layer; B - organic fertilization with manure: b₀ - 0 t/ha, b₁ - 50 t/ha, b₂ - 100 t/ha, b₃ - 150 t/ha; C - mineral fertilization with complex fertilizers: c₀ - N₀P₀K₀ kg/ha; c₁ - N₁₀₀P₈₀K₇₀ kg/ha; c₂ - N₂₀₀P₁₆₀K₁₄₀ kg/ha; c₃ - N₃₀₀P₂₄₀K₂₁₀ kg/ha (Şandor, 2011; Sabău, Şandor, 2013).

Taking in consideration that two plants, millet and spring wheat were used for phytoremediation, it was considered that the average annual yield differences (YD) between variants polluted with 3 % crude oil on the plowed layer and unpolluted, control variants could be used to assess the phytoremediation intensity. Because the intensity of the pollution with oil products can be evaluated against the production losses of the polluted plots compared to the neighboring, unpolluted plots, the evolution of the pollutant concentration during the research period can be evaluated indirectly by this variable, depending on the other studied factors (Toti et al., 2005).

Climatic characteristics of the research period, PS III-VIII (mm), MT III-VIII (°C) and dMAI III-VIII were calculated using data recorded at Meteorological Station Oradea located in the immediate vicinity of the experimental plots.

The statistical processing of the analyzed variables, the matrix of correlations and the analysis of the factors were made with the PSPP program, which is the free version of the SPSS statistical analysis program (PSPP Users' Guide, 2016). In order to test the significance of bilateral, linear, logarithmic and polynomial correlations, the Excel calculation module was used. In order to determine the form of bi-factorial equations of type $Y = f(X_1, X_2)$ and their statistical significance, the methodology indicated by Baghinschi, 1979 was used.

RESULTS AND DISCUSSION

Annual mean yield differences between the polluted controlled variants with 3 % crude oil and the control unpolluted variants (α_0 - α_1) being the majority negative are considered production losses of the polluted variants versus the unpolluted variants. These are between - 46.22 % and 17.57 %, the mean of the 10 years of research being - 20.54 %, affected by a standard error of the mean of 6.15% (Table 1).

Table 1

Statistical characterization of the variables that affect the oil phytoremediation from soil

Variables	n	Minimum	Maximum	Mean	Standard error of mean	Standard deviation	Variance
YD (%)	10	-46.22	17.57	-20.54	6.15	19.45	378.43
T (years)	10	1.00	10.00	5.50	0.96	3.03	9.17
PS III-VIII (mm)	10	212.60	536.60	377.06	37.15	117,49	13803.02
MT III-VIII (°C)	10	14.95	17.48	16.26	0.25	0.79	0.62
dMAI III-VIII	10	16.13	43.97	28.92	2.75	8.70	75.70

The criterion used to quantify the quantities of biodegraded crude oil through phytoremediation is the mean YD in percent, because during the 10 years two different crops of millet and spring wheat were used. The annual mean of YD in the first three years of the experiment when it was cultivated with millet was negative, ranging between -46.22 % and -33.69 % lower than those achieved by spring wheat grown in the last seven years, ranging from -30.62 % to 17.57 %.

The main production losses of the first three years of - 40.74 %, with a standard error of 3.70 %, were higher, than those obtained in the last seven years, of - 11.89 % characterized by a standard error of 6.12 %, justified by the fact that during this period, the oil concentrations from soil were higher (Fig. 1).

The analysis of the possible correlations between the studied variables, using the Pearson correlation coefficient suggests the existence of some direct links, statistically significant, between: dMIA and PS of the vegetation period (0.98); YD and T (0.91); PS and MT in the period considered (-0.73) and dMAI and MT respectively (-0.68), the latter two being inverse correlations (Table 2).

Correlations between dMAI and PS and MT respectively, during the months of March to August are explained by relationship used to calculate this index, which includes values of the two variables. These, together with

the reverse link between precipitation and temperature, are variables that characterize the climatic conditions of the area during the research period.

Since the assessment of the amount of crude oil biodegraded through phytoremediation is possible with the help of yield differences between the polluted and the unpolluted variant, their correlations with the other studied variables are of interest.

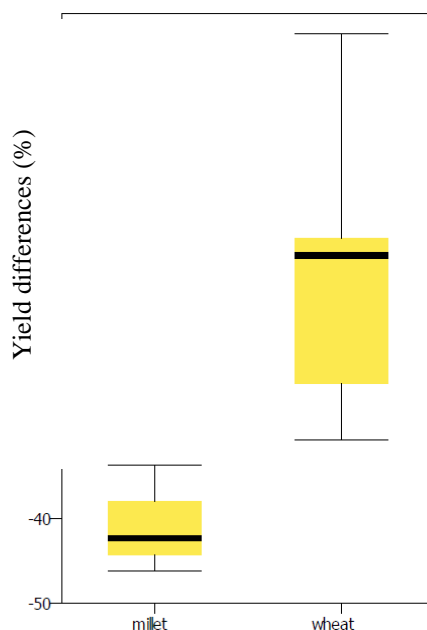


Fig. 1. The characteristic values of the main YD for the cultures used in the phytoremediation process

Table 2

Matrix of correlations between the analyzed variables

Variables	YD (%)	T (years)	dMAI III-VIII	PS III-VIII (mm)	MT III-VIII (°C)
YD (%)	1,00	0,91	-0,09	-0,06	0,30
Time (years)		1,00	0,07	0,09	0,35
dMAI III-VIII			1,00	0,98	-0,68
PS III-VIII (mm)				1,00	-0,73
MT III-VIII (°C)					1,00

From this point of view, the direct correlation (Pearson coefficient = 0.91) between YD (%) and T (years) indicates the increase from negative values at the beginning of the period (yield losses) to positive values at the end of period (yield gains)

Among the climatic factors considered, the strongest correlation (Pearson coefficient = 0.30) indicates the importance of MT from vegetation period on the YD and on the intensity of biodegradation process through phytoremediation.

The testing of the regressions of the YD, according to the time T, elapsed from the initiation of the experiment, indicates the existence of linear and distinctly significant logarithmic correlation, respectively very statistically significant polynomial of the second-degree correlations. The best correlation ratio is obtained by simulating YD by a second-order polynomial function, according to T, this being $R^2 = 0.8416$ (Fig. 2).

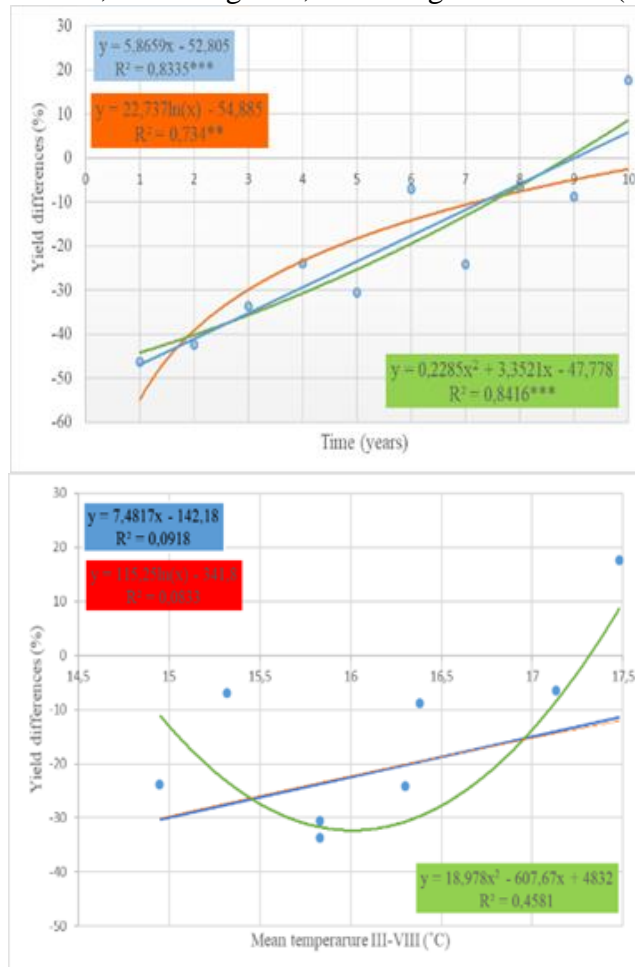


Fig. 2. Correlations of YD (%) versus T (years) and MT (°C)

In the case of the influence of the MT in the period III-VIII on the YD, quantified by the three types of correlations, it is noted that none of them have statistical significance, the closest to the limit of significance being the second degree polynomial link ($R^2 = 0.4581$).

The analysis of the main components by the correlation method shows that 83.01 % of the cumulative variance is explained by the correlation between the first two main components, the percentage YD and the T, duration of the experiment in years (Table 3).

By selecting the first three main components, in addition to the factors that represent the first two main components, the average temperature is added in the months of March to August, and thus the cumulative variance can be explained 98.90 %.

Table 3

Total variance explained							
	Variable Component	Initial eigen values			Rotation sums of squared loadings		
		Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1.	YD (%)	2.68	53.66	53.66	2.21	44.18	44.18
2.	T (years)	1.93	38.63	92.30	1.94	38.83	83.01
3.	MT III-VIII (°C)	0.33	6.60	98.90	0.79	15.89	98.90
4.	PS III-VIII (mm)	0.04	0.71	99.61			
5.	dMAI III-VIII	0.02	0.39	100.00			

The analysis of bi-factorial correlations of linear and second degree polynomial types, of the three selected factors, considering the YD as dependent variable, leads to very statistically significant, linear and polynomial correlations (Table 4).

Table 4

Bi-factorial correlations between YD (Y) and T (X1) and MT III-VIII (X2), respectively					
Equation	R ²	F calculated	F* p=5%	F* p=1%	Statistical Significance
$Y = -43.50 + 5.92 \cdot X_1 - 0.59 \cdot X_2$	0.83397	33.63	3.79	5.61	***
$Y = 2840.63 + 7.88 \cdot X_1 + 0.29 \cdot X_1^2 - 356.89 \cdot X_2 + 11.01 \cdot X_2^2 - 0.35 \cdot X_1 \cdot X_2$	0.94921	30.29	3.79	5.61	***

If we compare the determination coefficients made by the bi-factorial correlations $Y = f(X_1, X_2)$ and with one factor ones $Y = f(X_1)$, $Y = f(X_2)$ it is noted that both linear and polynomial second degree, these are higher for the bi-factorial ones.

The response surface of mean YD, according to T and MT, obtained using the second-order polynomial equation, with interaction between the two independent variables, indicates an upward trend in ID over T, because by phytoremediation, there has been a reduction in pollutant concentration from soil (Fig. 3).

The evolution of YD according to MT III-VIII indicates that biodegradation of the pollutant by phytoremediation is influenced by the climatic conditions during the experiments. Each year of the experimental period, the lowest values of the YD are obtained in the range of the minimum values of the MT of 16-17 °C, values, which increase for higher temperatures (18 °C) and respectively low ones (15 °C).

If high MT stimulates the crude oil biodegradation from soil, its stimulation when lower values of MT may be explained by the fact that they are correlated with increases in PS during the period, leading to the washing of the pollutant in depth and ensuring the necessary humidity of vegetative growth of crops.

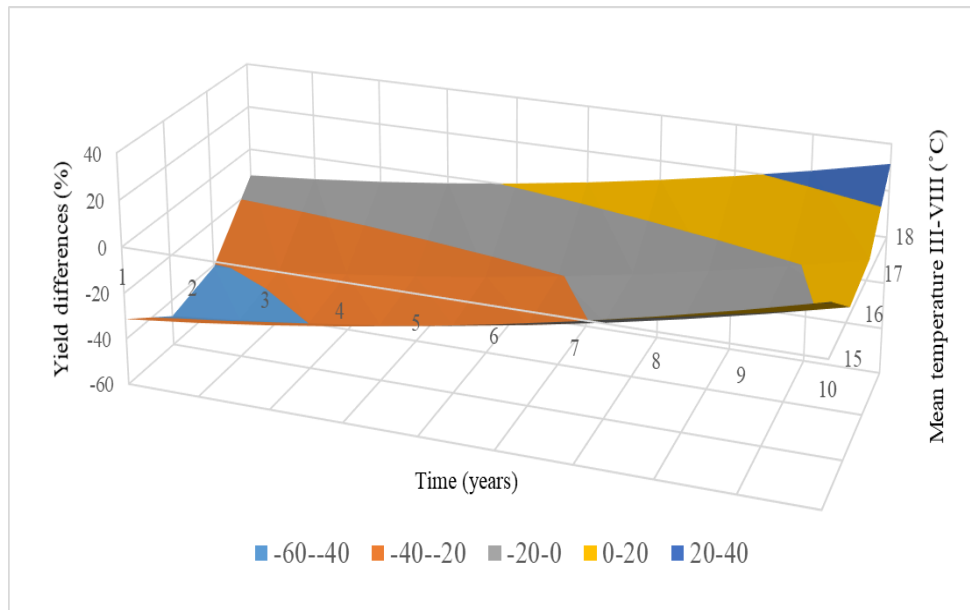


Fig. 3. The response surface of YD in function of T and MT

The term of the equation that indicates the interaction between independent variables T and MT show that the influence of MT on YD increases proportionally with the T, due to the reduction of the pollutant concentration.

Considering that in the case of soil pollution with crude oil, it is considered unpolluted for yield losses of up to 5 %, the polynomial equation thus established, allows the estimation of the period required for the ecological rehabilitation of the soil by phytoremediation. The period required for rehabilitation is obtained for the MT III-VIII value, making yield losses of 5 % or by nullification of YD.

Thus, it can be appreciated that under the conditions of MT III-VIII of 16 °C the biodegradation of the pollutant takes 8.5 - 9.5 years, whereas in the case of the MT of 18 °C this is reduced to 4.5 -5.5 years.

CONCLUSIONS

The average yield losses of millet, in the first three years of -40.74 %, with a standard error of 3.70 %, were higher than those obtained from

spring wheat in the last seven years, of -11.89 % characterized by the standard error of 6.12 %, due to the fact that at the beginning of the phytoremediation period the oil concentrations of soil were higher.

The correlations matrix between variables, that influence the pollutant phytoremediation reveals the direct correlative link between YD (%) and T (years), (Pearson coefficient = 0.91) indicating the increase from negative values at the beginning of the period (yield losses) to positive values at the end of the period (yield increases). Between the climatic factors considered, the strongest correlation link with YD is achieved by the MT III-VIII (Pearson coefficient = 0.30).

Bilateral correlations $YD = f(T)$ are distinctly significant for logarithmic function ($R^2 = 0.734$) and very significant for linear ($R^2 = 8335$) and second degree polynomial functions ($R^2 = 8416$). When connecting $YD = f(MT \text{ III-VIII})$, only the second degree polynomial function approaches to the significance ($R^2 = 0.4581$).

The analysis of the three main components show the fact that the connection between the YP, T and MT III-VIII represents 98.9 % of the total variance in the case of the studied factors.

The bi-factorial correlation, of the second-order polynomial type, with the factor interactions, between the yield losses and T and MT III-VIII, respectively, statistically very significant, allow the determination of the duration of the biodegradation of the crude oil from soil, by phytoremediation, in relation to the MT during the vegetation period cultures. This duration is 8.5 – 9.5 years for the MT of 16 °C, whereas in the case of a MT of 18 °C this is reduced to 4.5 – 5.5 years.

REFERENCES

1. Ardelean M., Sestraș R., Cordea M., 2005, *Tehnică experimentală horticolă*. Academicpres Publishing House, Cluj-Napoca
2. Atlas R.M., 1981, Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbial Rev.* 45(1), pp.180
3. Baghinschi V., 1979, *Funcțiile de producție și aplicațiile lor în agricultură*. Ceres Publishing House, Bucharest, pp.240
4. Banks M.K., Schultz K.E., 2005, Comparison of plants for germination toxicity tests in petroleum - contaminated soils. *Water, Air and Soil Pollution* 167, pp.211-219
5. Colibaș I., Colibaș M., Șandor M., 1995, Măsuri de ameliorare a solurilor poluate cu rezidii petroliere. Cum să cultivăm pământul în zona centrală din vestul țării, *Stațiunea de Cercetări Agrozotehnice Oradea*, pp.109-111
6. Elsner M., Schwarzenbach R.P., Haderlein S.B., 2004, Reactivity of Fe (II)-bearing minerals toward reductive transformation of organic contaminants. *Environ. Sci. Technol.*, 38, pp.799
7. Gkorezis P., Daghio M., Franzetti A., Van Hamme J.D., Sillen W., Vangronsveld J., 2016, The interaction between Plants and Bacteria in the Remediation of

- Petroleum Hydrocarbons: An Environmental Perspective. *Front Microbiol.*, 7, pp.1836
8. Glick B.R., 2010, Using soil bacteria to facilitate phytoremediation. *Biotechnology Advanced*, Vol. 28, Issue 3, pp.367-374
 9. Ichim M., Vişan A., Enache R., 2010, Phytoremediation of lead contaminated soils. 3rd International Symposium of Biotechnology, Bucharest
 10. Muratova A.Y., Golubev S.N., Dubrovskaya E.V., Pozdnyakova N.N., Panchenko L.V., Pleshakova E.V., Chernyshova M.P., Turkovskaya O.V., 2012, Remediating abilities of different plant species grown in diesel-fuel-contaminated leached chernozem. *Appl Soil Ecol* 56, pp.51-57
 11. Nie M., Wang Y., Yu J., Xiao M., Jiang L., Yang J., Fang C., Chen J., Li B., 2011, Understanding plant-microbe interactions for phytoremediation of petroleum polluted soil. *PLoS One* 6:e17961
 12. Răducu D., Constantin C., Eftene M., Eftene A., 2012, Oil Components Distribution in Soil - Micromorphological Approach. *Natural Resources and Sustainable Development*, vol. 2, pp.105-108
 13. Sabău N.C., Şandor M., 2013, The Influence of the Fertilization Systems on Production in the Last Years of Agrochemical Improvement of a Crude Oil Polluted Soil. *Natural Resources and Sustainable Development*, Vol. 3, pp.415-422
 14. Sabău N.C., Şandor M., 2014, The influence of fertilization on wheat yield losses achieved during the agrochemical melioration of a soil, under control polluted with crude oil, from Oradea. *Annals of the University of Oradea, Fascicle Environmental Protection*, Vol. XXIII, 2013/B, pp.763-768
 15. Sabău N.C., Şandor M., 2015, Correlations between the yield differences on spring wheat obtained on a polluted haplic luvisoil with crude oil, depending on weather conditions and fertilization systems on the mitigation period. *Natural Resources and Sustainable Development*, Vol.5, pp.143-154
 16. Săulescu N.A., Săulescu N.N., 1967, *Cîmpul de experiență*, 2nd edition. Agro-Silvică Publishing House, Bucharest
 17. Şandor M., 2011, *Poluarea solurilor cu petrol, în 50 de ani de cercetări agricole în Oradea*, Fascicula I. Culturi de câmp și Furajere, coord. Domuța C., University of Oradea Publishing House, pp.476-500
 18. Şandor M., Brejea R., Domuța C., 2013, Research Regarding the Reconstruction of the Polluted Soil with Oil from Suplacu de Barcău, Romania. *Natural Resources and Sustainable Development*, Vol. 3, pp.423-432
 19. Toti Mh., Dumitru Mh., Voiculescu A.R., Mihalache Mh., Mihalache G., Constantinescu C., 2003, *Metodologia de biodegradare a solurilor poluate cu țitei, cu ajutorul microorganismelor specifice selecționate din microflora autohtonă*. GNP Minischool Publishing House, pp.164
 20. Urs A., Micle V., 2010, *Studiu privind proiectarea sistemelor de bioremediere a solurilor contaminate*. The 10th Multidisciplinary National Conference "Professor Dorin Pavel" – founder of Romanian hydroenergetics, Sebeș, Alba
 21. Wang Zhen-Yu, Xu Ying, Wang Hao-Yun, Zhao Jian, Gao Dong-Mei, Li Feng-Min, Xiang B., 2012, Biodegradation of crude oil in contaminated soils by free and immobilized microorganisms. *Pedosphere*, vol. 22(5), Published by Elsevier Ltd., pp.717-725
 22. ***, 2016, *GNU PSPP Statistical Analysis Software, PSPP Users' Guide*

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