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LEAF FRACTAL DIMENSIONS IN ECOTOXICOLOGICAL ASSESSMENT

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Abstract
Toxicity testing represents one of the methods used in environmental technology with predictability in risk assessment. These have a great deal of efficiency in quantification of pollutants after its interaction with the biotic and abiotic environment as integral parts of an ecosystem. The study was conducted by assessed response of plants (Populus spp.) to phytotoxic compounds which can be interpreted by morphological and physiological parameters of test organism. Leaf length and, especially, fractal dimension are shown to be good indicators of plant response to toxicants in their environment. The source of toxic pollutants was landfill leachate the composition of which is very complex with a high degree of toxicity because it contains a diversified spectrum of heavy metal ions, ammonia, persistent organic pollutants or xenobiotic compounds. Plants were exposed to landfill leachate solutions. Phytotoxicological experiments were conducted during 4 weeks on the cuttings poplar (Populus spp.) using five successive concentrations of landfill leachate (6,25%; 12,5%; 25%; 50%; 100%), in triplicate and keeping the control of process with tap water representing 0% reference solution. The tolerance of plants was determined by quantification of morphological parameters of leaves including their fractal dimension simultaneously with to determine the range of values (LOEC) the lowest effective concentration causing toxic effects and (NOEC) no-toxic effect concentration. Digital images have been analyses using softwares in close correlation with the leaf fractal dimensions and the following leaf parameters: length, width, area, weight. It can be observed that with the increase of landfill leachate concentration determined increase in fractal dimension (Df) of cuttings poplar leaves.

Keywords: fractal dimensions, landfill leachate, phytotoxicity, pollutants, poplar.

1. INTRODUCTION

In environmental technologies ecotoxicity tests are required. These studies have a high performance through the ability to characterize an environmental situation and additionally to plan a reconstructive or remediative method of a polluted ecosystem.

The foliar surface architecture can be considered a synthetic bioindicator for the plants with the ability to assess morpho-functional toxicity (Fodor and Hâruță, 2008). On the whole, dimension can be defined as the number of coordinates or parameters that is used for describing geometrical objects or organisms.

Traditionaly, Euclidean geometry describes the basic regular forms such as lines, squares, cubes (Mandelbrot, 1977; Mandelbrot, 1982). The fractal geometry can analyze complex forms: geometric (regular) and non-geometric (irregular). A geometric fractal is based on of large and small structures that resemble precise duplication of each other. And in irregular fractals, there are also large and small structures, but they do not resemble to each other (Campbell, 1996). Commonly, lines are defined as one dimensional, while squares are two dimensional. Fractals are irregularly

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shaped objects formed of repeating similar model. They cannot be described with a whole number dimension. Mostly, in nature, objects such as the outline of leaves, trees, and coastlines are fractal and are between one and two dimensional (Bayirli et al., 2014).

The fractals of leaves are a repeatedly dented or deformed circle. Fractal analysis has been used as an accuracy method with increased predictability to characterize the complexity of plant structures. Leaf morphology has been studied since the origin of botany as a science, by using terminology to describe the edge of the leaves as being linear or jagged and leaf shapes, such as obovate, ovate, oblong, needle-shaped, lanceolate (Ernesto et al., 2010).

The diversity of shapes in nature, as well as the ambiguity of terms used in describing structures it difficult to clearly define and use these shapes as taxonomic characters. Nevertheless, it is necessary a comparative analysis of nonhomologous structures. In this context, biometry reprezent better solution to describe and analysis leaves, thus avoiding ambiguous terms for their characteristics. As a consequence, can be a remarkable development in identification and determination of species enabling the use of this method not only in botany studies, but also in related fields such as forestry or paleobiology (Mouton, 1976).

Fractal dimension (Df) has been used to characterize the complexity of a structure by quantifying irregular aspects in biometric studies of plant morphology. Elements with a low complexity have fractal dimensions close to their topological dimension, meanwhile, elements with a high complexity have a tendency to cover the spatial region that they define. In close correlation with this tendency, represented by the fractional values of the fractal dimension is to the complexity of the dynamic formation of the structure, an important topological attribute used for its characterization (Mandelbrot, 1982; Chaves, 1989; Weibel, 1991).

Transformations induced by toxic on living tissues take on account histo-morphological changes as well as physiological disturbances experienced throughout the living organism (Pohonțu et al., 2016). Thus, by fractal dimension has been quantified and graded the complexity of these structures. Different pluricellular plant organisms have been used over time to quantify the tolerance to phytotoxic compounds (Dimitriou et al., 2006; Bialowiec et al., 2010).

The morphological response of plant organisms in phytotoxicity testing takes into account: rate of seeds germination, necrosis, chlorosis, or reduction of surfaces of some tissue formations most often at the leaf level. Wiping may be considered another histo-morphological indicator, synonymous with mortality from animal organisms taken in toxicity tests at different substances and doses.

All morphological changes in the plants cause physiological imbalances, among which we mention the reduction of the photosynthetic, the assimilation and the growth rate or decrease of the evapotranspiration process (Bialowiec and Raderson, 2010). Bio-indicative phytotoxicity models can be applied on a large scale to a multitude of plants, higher or lower toxic tolerance on the majority of pollutants or xenobiotic compounds, including landfill leachate (Bialowiec et al., 2010b).

Landfill leachate can be considered a liquid waste, a mixture with complex composition generated from rainfall water that crossing through wastes stored in open space (Pohonțu, 2011). By its ammonia and heavy metal ions content, landfill leachate is a solution with high toxicity (Reghab et al., 2013).

The assessment of woody plants response to potentially toxic substances in landfill leachate were taking into account: negative influence of landfill leachate on willow and regime of exposure to landfill leachate which may influence tolerance to phytotoxic compounds. The box fractal dimensions of leaves is an indicator of woody plants response to landfill leachate, which can be correlated with other morphometric parameters of leaf (Bialowiec et al., 2010).

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The aim of this study was to assess the toxic effect of multiple pollutants from landfill leachate on the fractal dimensions of poplar leaves.

2. MATERIALS AND METHODS

The experimental procedure consisted of interaction between biotic and abiotic component, outside, under the weather conditions of June 2017: temperature in range of 25 °C ± 5 °C, relative atmospheric humidity 70% ± 8% and natural dark-light 9 to 15 hours. Thus, morphological responses were obtained following exposure to various toxic doses of cuttings poplar and their quantification by fractal dimensions.

Populus spp. cuttings having approximative 20 – 25 cm long and 0.5 – 1 cm diameter, provided from intensive poplar culture Dorneşti – Suceava. All cuttings derived from one poplar plant, for the accuracy of the results sought and for the reduction of genetic diversity.

The source of toxic pollutants were two different types of landfill leachate: stabilised coming from an older waste disposal site Botoşani and other source of unstabilised from Iaşi, a relatively new landfill. Both sources have a very complex composition with a high degree of toxicity because it contains a diversified spectrum of heavy metal ions, ammonia, persistent organic pollutants or xenobiotic compounds, highlighted in table 1.

Table 1 Initial characteristics of both phytotoxic sources

Parameters	***Pollutant sources***	
	Unstabilised landfill leachate	***Stabilised landfill leachate***
Reaction (pH)	8.6	7.8
Electrical conductivity (mS/cm^{-1})	19.7	51.5
Chemical Oxygen Demand (mg O_2/L)	2875	4773
Ammonium (mg/L^{-1})	545	2079
Phosphorus (mg/L^{-1})	9.5	11.8
Fixed residue (mg/L^{-1})	459	903

Phytotoxicological experiments were conducted during 4 weeks on the cuttings poplar (*Populus spp.*) immersed in five successive dilutions of landfill leachate such as: 6,25%; 12,5%; 25%; 50%; 100%, in glass bottles, triplicate for each concentration. These were the independent variables of the process. The control of process has been achieved with tap water representing 0% reference solution.

The tolerance of plants was determined by quantification of morphological parameters of leaves including their fractal dimension simultaneously with to determine the range of values LOEC (the lowest effective concentration causing toxic effects) and NOEC (no-toxic effect concentration). Digital images have been analyses using softwares in close correlation with the leaf fractal dimensions and the following leaf parameters: length, width, area, weight. These were the dependent variables of the process. Mean values of determined leaf parameters were fitted with the ANOVA at the significance level of $p < 0.05$.

To calculate fractal dimensions of leaves, obtained after the 4 weeks of vegetation, were scanned into a computer at 300 DPI resolution as black and white saved bitmap files. The images thus obtained were digitally analysed using the software Image Tool for measuremet length and width of leaves or Image J software for determination of leaf area. Leaf weight was determined with Panther AS 160.R2 analytical balance.

Fractal dimensions was then used to analyze each leaf image, depending on the toxic concentration at which they were exposed. The fractal dimension (Df) was prformed using the box-counting

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method (Feder, 1988). By the software Benoit were calculated the number of boxes of a certain size needed to fill the space around an image. This process was applied to each leaf. Beginning with an initial size of two, the box size was slowly increased by intervals of two until twenty was reached. The program then displays a log-log graph of the box size versus number. Fractal dimension then found the slope of the best fit straight line, which is the fractal dimension of the leaf.

For phytotoxic response evaluation was calculated inhibition rate in accordance with equation number 1 (Bialowiec and Raderson, 2010) (Bialowiec et al., 2010).

$$I = 100\frac{(C - T)}{C} \quad (1)$$

where,

I – effect caused by each landfill leachate concentration (%);

C – mean value of measured parameter for reference variant with tap water;

T – mean value of measured parameter for each landfill leachate solution.

The lowest effective concentration causing toxic effects (LOEC) was determined using the function number 2 (Bialowiec and Raderson, 2010) (Bialowiec et al., 2010).

$$I = a_1 - Exp^{(LOEC - a_2 - Cr)} \quad (2)$$

where,

Cr – landfill leachate concentrations used in the experiment (%);

a_1 and a_2 – function parameters estimated by regression.

3. RESULTS AND DISCUSSIONS

Based on the above-mentioned working protocol, the morphological parameters, interpreted by biometric indices, were analyzed, depending on the toxic concentration at which the poplar cuttings were exposed. According to equation number 1 and 2 was calculated the lowest effective procentual concentration of landfill leachate causing toxic effects. Thus, landfill leachate concentration values exceeding the concentration expressed by LOEC, inhibit the normal vegetative processes quantifiable by foliar size, fractal dimension and leaf weight corresponding to each level of pollutant concentration.

Table 2 LOEC values of different determined parameters

Biometric parameters	*Unstabilised landfill leachate*	*Stabilised landfill leachate*
	LOEC (%)	*LOEC (%)*
Leaf length	7.15	6.28
Leaf width	6.82	6.01
Leaf area	7.08	6.97
Leaf weight	6.79	5.73
Fractal dimesion	5.91	5.52

As can be seen in Table 2, stabilized landfill leachate exhibits higher phytotoxicity than unstabilized leachate for all analyzed biometric parameters. In this context, vegetative processes of growth and development that occur in poplar cuttings, being inhibited at lower concentrations of landfill leachate. This can be explained by the presence in stabilized landfill leachate of persistent organic pollutants, which are non-biodegradable, compared to the unstabilized landfill leachate whose organic fraction is biodegradable and simultaneously with a lower ammonium concentration.

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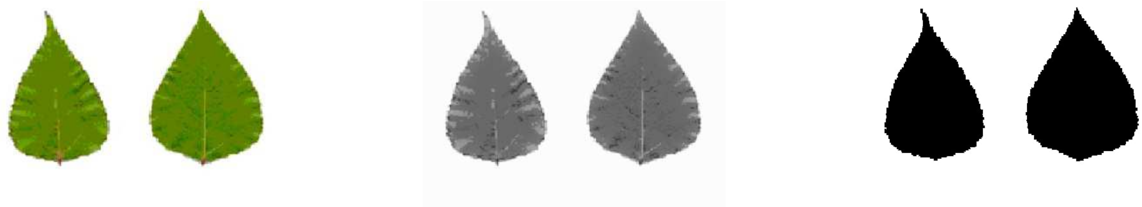

Figure 1 Poplar leaves with different fractal dimesions (Df) but the same length during the digital analysis

In figure 1, can be observed the difference of area and fractal dimensions between two poplar leaves with the same length, but which have been exposed to various phytotoxic concentrations of landfill leachate.

Agreed with those previously expressed, were studied comparatively, ecotoxicological responses, depending on the source and the origin of the pollutant. For all the morphological parameters determined, at 50%, respective 100% concentrations did not give any vegetative response, due to the strong toxic effect.

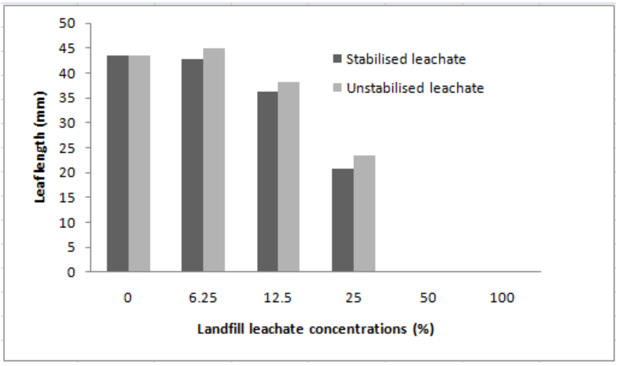

Figure 2 Leaf length

Thus, in figure 2 the foliar elongation values are illustrated, where it is observed that the toxic effect increases with the increase of the applied pollutant concentration. The ecotoxicological response manifests by reducing leaf length. The exception, however, is that the leaves in whose saplings were applied unstabilised leachate, with a high organic content, only at the concentration of 6.25%.

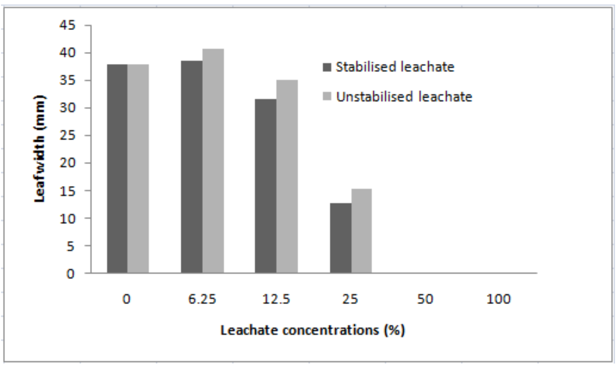

Figure 3 Leaf width

Morphological parameter, leaf width, described in figure 3, shows that the leaf width reduction compared to the 0% blank sample is for both types of pollutant only at concentrations of 12.5% and 25%. At the last of the concentrations (25%) for which the vegetative response was received, the leaf width is reduced by more than half compared to the 0% blank sample.

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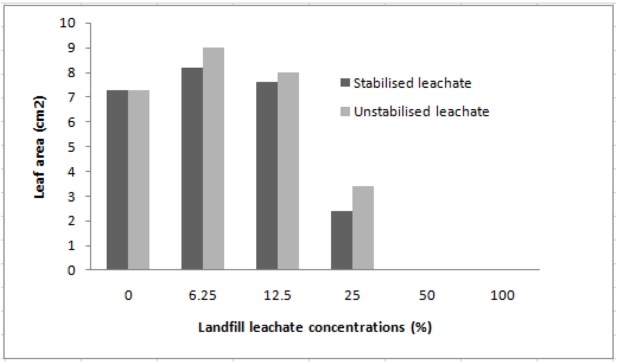

Figure 4 Leaf area

In figure 4, it is found that both the pollutant concentration of 6.25% and the concentration of 12.5% for any leachate types, stabilized and unstabilized, the leaf area increases compared to the 0% blank sample where used only tap water. Which indicates that at these concentrations for the determined parameter, leachate does not show toxic effect.

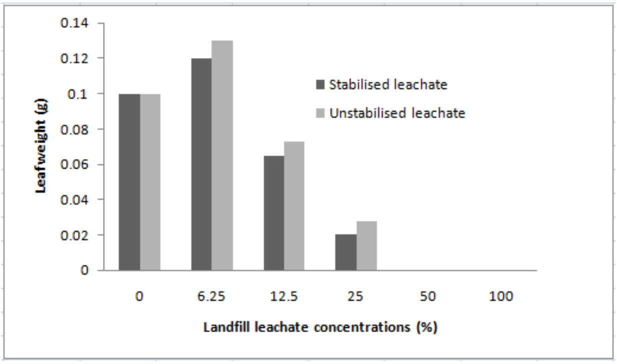

Figure 5 Leaf weight

According to figure 5, it is observed that at pollutant concentrations of 6.25% for both types of leachate, leaf weight is much higher than the control sample, 0% pollutant, so this concentration does not show any toxic effects, which indicates that it may be like a fertilizer by the present nitrogen of its composition. Leaf weight is reduced to 25% pollutant concentration by more than 2/3 compared to 0% concentration.

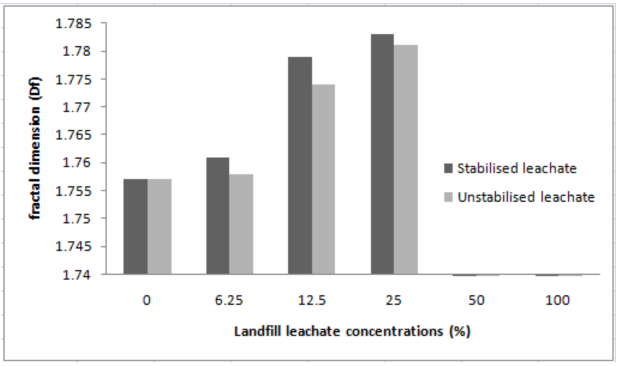

Figure 6 Fractal dimension

As can be seen in figure 6, fractal dimensions (Df) increases at the same time as the increase of the applied pollutant concentration. Therefoe, Df values, estimated with box-counting method, varied between 1.761 to 1.783 for stabilised leachate and 1.758 to 1.781 for unstabilised leachate, in range

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of concentrations 6.25% - 25%. Higher values of Df as reported at stabilised landfill leachate. Nevertheless, there were no significant ($p < 0.05$) differences between leaves of poplar who grew up in both sources of pollutants, at concentrations of 6.25%, but a significant ($p < 0.05$) ascending relationship of Df mean values was observed in the range of leachate concentration from 12.5% to 25%. Higher difference between the Df values for the two leachate sources was recorded at the 12.5% concentration. The highest value for Df was determined for the poplar leaves exposed to the concentration of 25% unstabilized leachate, and the lowest value for Df, nearing the value obtained at the control reference sample, was determined at the concentration of 6.25% for the poplar leaves exposed in unstabilized leachate.

For all the situations described, in correlation with the source of the pollutant, more precisely with its degree of stabilizing, it can be noticed that the old and stabilized leachate due to the elimination of the biodegradable organic compounds fraction, the higher ammonium content and the presence of the persistent pollutants, exhibits a higher toxicity effects compared to unstabilized leachate, which has yet biodegradable organic compounds in its composition.

4. CONCLUSIONS

The present paper highlights how the functional and morphological parameters of poplar leaves, *Populus spp.*, can be affected at different stages of development and depending on the amount of pollutant applied.

Thus, the leachate toxic capacity on vegetal tissues was observed in vegetation processes of poplar cuttings, concomitant with the bioindicating capacity of the leaves.

The dose-response curve indicates that with increasing of landfill leachate concentration also increases the response of the vegetal tissue concretized through similar toxic effects in each determined morphological parameters – decrease of poplar leaf length, width, area, and weight. Exceptions made the range of landfill leachate concentrations between 6.25% to 12.5%, at which an increase in biometric parameters of poplar leaves was observed, indicating that at low leachate concentrations, it functions as a fertilizer by the high nitrogen content.

With increasing of landfill leachate concentration is observed increase in fractal dimension (Df) of cuttings poplar leaves. Df values varied in range of 1.761 - 1.783 for stabilised leachate and 1.758 - 1.781 for unstabilised leachate, the lowest value being encountered at low concentrations of pollutant, and the highest value at elevated concentrations of pollutant applied.

Poplar cuttings exposed at 100% and 50% landfill leachate concentrations of both sources did not give any vegetative response.

Regarding pollutant sources, it has been found that stabilized leachate from older landfills produces more toxic effects compared to unstabilized leachate from recent landfills.

Determination of fractal dimensions (Df) in phytotoxicity tests is therefore a rapid, efficient method and a good indicator for testing toxicity in a diversified spectrum of substances.

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