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Using Statistical Modeling for Assessing Lettuce Crops Contaminated with Zn, Correlating Plants Growth Characteristics with the Soil Contamination Levels

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Abstract: The aim of the study was to identify new mathematical models and strategies that can characterize the behavior of pollutants accumulating in the soil over time, considering the special characteristics of these chemicals that cannot be degraded or destroyed easily. The paper proposes a statistical model for assessing the accumulation of Zn in the lettuce (*Lactuca sativa* L.), based on three indicators that characterize the development of lettuce plants over time. The experimental data can be used to obtain interpolated variations of the mass increase functions and to determine several functions that express the time dependence of heavy metal accumulation in the plant. The resulting interpolation functions have multiple applications, being useful in generating predictions for plant growth parameters when they are grown in contaminated environments, determining whether pollutant concentrations may be hazardous for human health, and may be used to verify and validate dynamic mathematical contamination models.

Keywords: heavy metals; soil contamination; zinc accumulation

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1. Introduction

Zinc (Zn) reaches the agricultural soil through anthropogenic activities such as the application of fertilizers and pesticides, manures, sewage sludges, or various industrial activities, in many cases causing phytotoxic concentrations or contaminating agricultural products [1]. High concentrations of heavy metals in agricultural soil could generate toxic effects on human health, and their negative effect is aggravated by the property of metals to accumulate in time, producing chronic intoxication [1]. Although zinc is an important nutrient, its excessive ingestion reduces the immune functions and the level of high-density lipoproteins. Food consumption is the main pathway for human exposure to heavy metals [2], and zinc has one of the greatest accumulation potentials in plants [3]. A study [4] showed that the potential of heavy metals to be absorbed in plant parts is higher in roots, followed by stems and leaves.

Given the high-risk potential caused by the presence of heavy metals in the soil, both from the perspective of ensuring food security and human health, the accumulation of various hazardous substances in the mass of plants has been an intensely studied topic. Recent progress in the research of toxic metals and their interactions with essential elements has increased our understanding of the mechanism of toxicity at the biochemical level. Boskovic-Rakocevic studied the total Cd levels and the accumulation process in lettuce in order to evaluate the human health impact [5], determining that the uptake and accumulation of cadmium is mostly affected by soil pH, followed by Cd availability. A study [6] evaluated the biochemical effect and the physiological response of several contaminating agents in peppers raised in laboratory conditions, while other research [7] evaluated Zn phytotoxicity considering the complex biochemical reactions, and plant tolerance to Zn with regard to phytoremediation processes.

In a paper [8], the natural Cu, Cr, Zn, and Mn phytoaccumulation potential was screened for three widespread species, in the Constanta coastal area, Romania: Brassica rapa, Crambe maritima, and Lepidium draba. Samples of aboveground organs and soil were taken for each species and analyzed for heavy metal concentration values. Regarding the statistical analysis of the content of heavy metals in the soil, studies [9] evaluated the accumulation of cadmium and lead in vegetables that are grown in contaminated soils. The comparative analysis concluded that cadmium accumulation by plants grown on sewage sludge amended soils is lower than that for the inorganically contaminated soils. An extensive study on a total of 118 agricultural soil types and 43 vegetable samples have been developed in [10], assessing the spatial distribution, sources, accumulation characteristics, and potential risk of heavy metals in the agricultural soils and vegetables, by three different approaches. Contamination with heavy metals can also come from the water used to irrigate crops, as shown in a study [11] that considered the effect of wastewater irrigation on vegetables, in relation to the bioaccumulation of heavy metals and biochemical changes. The results showed a decrease in total chlorophyll and total amino acid levels in plants and an increase in the amounts of soluble sugars, total protein, and ascorbic acid in plants grown on soils irrigated with contaminated wastewater.

The statistical analysis is widely used in modern research, being the best approach for primary processing of data resulting from the investigation of phenomena. Statistical modelling is not only a way to obtain conclusive results, but also an intermediate step towards analytical, deterministic, or probabilistic models, with a greater degree of generality. In terms of plant evolution, and the bioaccumulation of contaminants in plants, a complex multivariate analysis on the presence of heavy metal contamination in agricultural soils is analyzed in a study [12]. The experimental results can be presented in statistical terms, even in terms of simple statistical models, such as the linear regressions [13]. Linear and quadratic regressions are often used in investigations envisaging the interaction of heavy metals with plants [14].

A study [15] showed a detailed analysis of the mathematical models generally used in ecology, classifying them into deterministic and statistical models. The bioaccumulation assessment uses both types of models for prediction or optimization, or for deepening various aspects. Despite the development direction that emphasizes the need to know the causality of the processes underlying the bioaccumulation processes, the use of statistical models for prediction is an extremely valuable asset [15].

To compare different types of components or the effects produced by different techniques or technologies, statistical research frequently uses the analysis of variance, or ANOVA [16–20].

Superior statistical techniques are currently employed to predict the heavy metal concentration in soil, plants, or other environments, in the frame of complex computational tools such as the neural networks [21–23].

This paper presents one-dimensional interpolations, regarding both the variation over time of the mass of lettuce plants grown on soil contaminated with zinc, and the variation of heavy metal concentration in plants. It also includes two-dimensional interpolations regarding the variation of the mass of plants (lettuce) that are grown on soil contaminated with zinc, depending on time and the amounts of zinc in the soil.

The experimental studies aimed to create a framework for a better understanding of the mechanisms of Zn toxicity in plants and to identify novel assessment perspectives based on a statistical analysis. The physiology of Zn phytotoxicity is complicated, considering Zn interference in chlorophyll biosynthesis, and other complex biochemical reactions [7]. There are different tolerances to zinc toxicity, depending on plant genotypes, with lettuce being one of the plants with higher tolerances.

The aim of this paper was to identify new tools to describe how zinc pollution affects lettuce crops, analyzing three growth characteristics: plant mass, plant height, and plant diameter. Using the least squares method on the experimental data has determined the 1 to 4 grades mathematical regression functions that describe the three growth characteristics in time. The three plant growth characteristics (mass, height, and diameter) have been correlated with the three zinc concentrations in the soil, in order to draw conclusions about the effect associated to each concentration on the lettuce experimental culture.

2. Materials and Methods

This research study has three components: the physical component, the experimental component, and the numerical component, consisting of the results of the descriptive statistical analysis of the experimental data. The research has been performed in two phases: the experimental phase and the processing of statistical data, followed by the interpretation of the results. Statistical processing led us to the elementary statistical models of the process of zinc bioaccumulation in the bio-plant bio system.

The experimental design started from the scientific problem of determining the effect that different zinc concentrations may have on lettuce plants and implicitly on human health, using statistical techniques. The evaluation method was to determine the interpolation functions, that can be used for making forecasts on the evolution over time of plant development, in soils contaminated with zinc.

The experimental unit was the plant isolated in individual pots. A single treatment was applied to the soil, followed by the monitoring of the evolution of the plant development in time. The factor has been considered that the zinc solution has three different levels (three different concentrations of zinc applied to the soil).

The response (the outcome being measured) was the plant development in the 10 weeks of monitoring. The period of 10 weeks was chosen, because it was considered the maximum until harvest should be done for human consumption. Three observational units were analyzed, namely the height of the plant, its mass, and plant thickness. The treatment stage did not change after applying the three concentrations of zinc on the soil, and the growing conditions remained the same. The resulting design considered comparisons between samples.

A completely randomized design was used, randomly assigning treatments to the experimental units in a pre-specified number, with the same number of units receiving each treatment.

The experimental method involved the use of 30 lettuce plants in individual pots, forming three groups of 10 pots. Each group was initially infested in a controlled manner with a certain level of contamination (initial concentration of zinc). Lettuce seedlings were planted on clean soil, and then contaminated with three solutions containing the following zinc concentrations: 1.5%, 3.0%, and 4.5%. Contamination was carried out by adding Zn sulphate solutions (250 mL solution, for each zinc concentration) on clean soil (1 kg/pot of fertile soil). The physicochemical properties of the control sample (i.e., of the uncontaminated soil used for the growth of all plants, which was then infested with Zn solution in each of the three groups of 10 pots) were as follows: the pH varied between 6.0–7.0; total nitrogen 1.9%; total phosphorus 0.5%; potassium 0.9%; electrical conductivity 1.2; maximum 5% soil particles over 20 mm; humidity 14.7%.

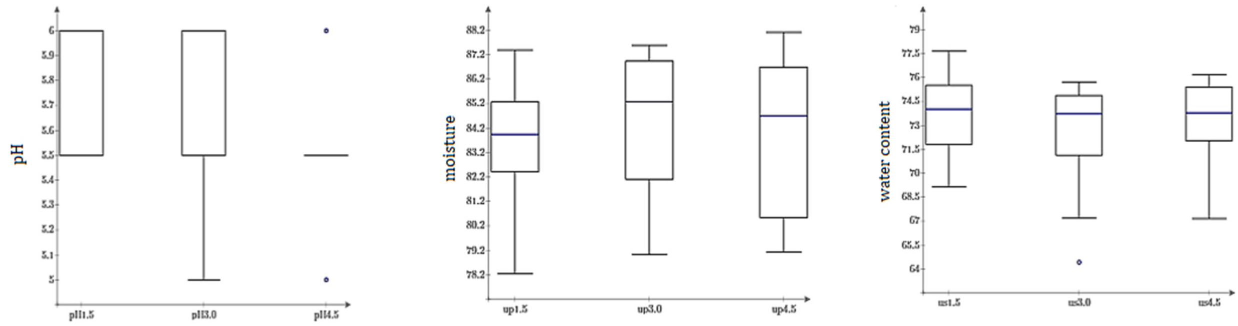
At each harvest, three plants were sacrificed, one from each group of 10 pots with different initial concentrations of Zn. For each plant harvested, the mass, height, diameter, and zinc concentration of the plants were measured for each of the three samples. At each harvest, the parameters were measured: zinc concentration in the soil, soil moisture, water content of the plant, and pH. Ten harvests were made from October to December at one-week intervals. The culture was located in a greenhouse with controlled air temperature and humidity (temperature 20 °C and humidity 65%). Four experimental parameters were measured at each harvest and introduced in the statistical analysis (mass, height, crown diameter, and zinc concentration).

The soil–plant biological system is simplified in this empirical-statistical model by the elementary dependence of the output parameters, on a single input parameter (zinc concentration in the soil in which lettuce grows), and time:

$$x = f(t, c) \tag{1}$$

where: x is one of the output parameters (plant mass, crown diameter, plant height, zinc concentration in the plant, the water content of the plant, or soil moisture, soil pH, and the amount of zinc in the soil), c is the zinc concentration from the soil in which the plant grows, and t is the time.

The physicochemical characteristics (pH, soil moisture, and plant water content) of the soil varied in relatively narrow ranges, as it can be seen in Figure 1.



(a) (b) (c)

Figure 1. Box plot representations of the distribution of pH (**a**), soil moisture (**b**), and plant water content (**c**), during the experiment, for the three cases of soil contaminated with a solution of 1.5%, 3.0%, and 4.5% zinc concentration.

Plant and soil sampling and analysis were performed daily, up to 68 days after planting (Figure 2). The mass of the samples was determined by weighing the probes with the KERN electronic balance (0.001 g precision), and plant height from the root levels to the top of the plant was measured with a ruler.

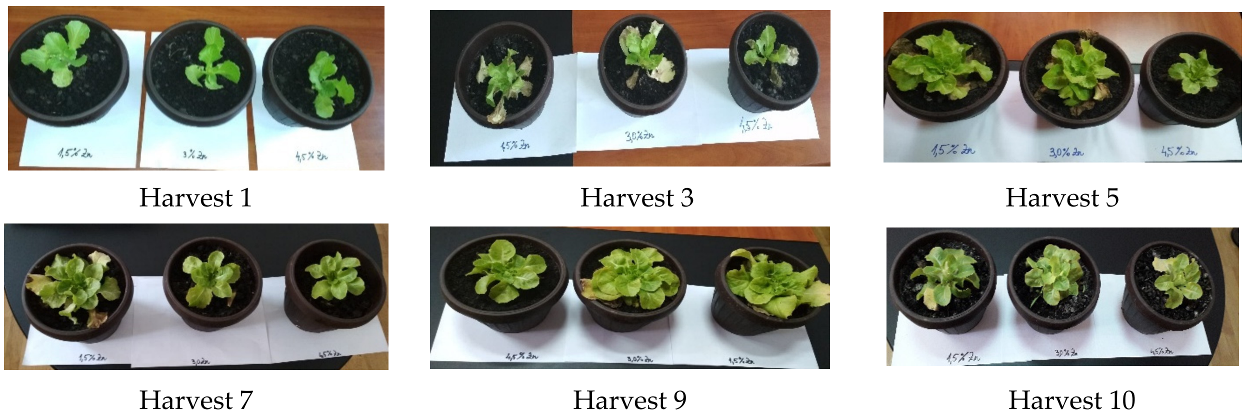
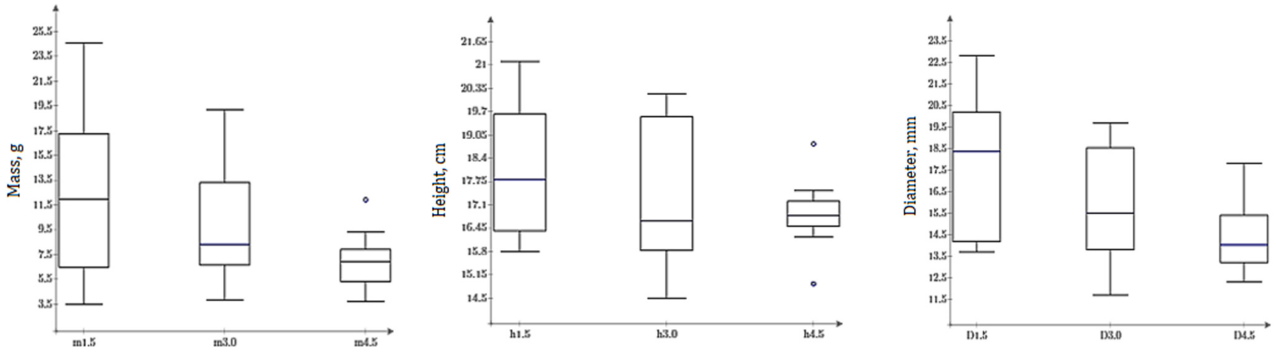


Figure 2. Plants with different contamination concentrations, harvested at different time periods after cultivation.

Soil moisture and water content, both in the plants and soil, were determined by drying in the oven at 105 °C, in order to evaporate water both from the soil and from the plant.

Soil pH was determined using a pH determination kit. A soil sample of 20 g was dried in the oven and then placed in a bowl with 100 mL of distilled water, stirred for 30 min and then filtered and controlled with pH paper.

An overview of the evolution of the mass, height, and diameter for the last 10 harvests of lettuce grown in the three soil types (each being contaminated by adding, in the soil, three solutions having 1.5%, 3.0%, and 4.5% zinc concentrations), is given by a box plot representation in Figure 3.



(**a**) (**b**) (**c**)

Figure 3. Box plot diagrams for mass (**a**), height (**b**), and diameter (**c**) distributions for the three cases of soil contamination with a solution of 1.5%, 3.0%, and 4.5% zinc concentration.

The determination of zinc in the contaminated soil and the lettuce plant body (root and leaves) was performed by the spectrophotometric method (flame atomic absorption), according to the procedure developed in a previous study [24].

Figure 4 shows a representation of experimental data obtained by normalized standard deviation, depending on the Zn concentration in the soil. It can be observed that the "tightest" results around the average, in terms of mass, height, and diameter of the plant, are obtained at the highest concentration of zinc in the soil.

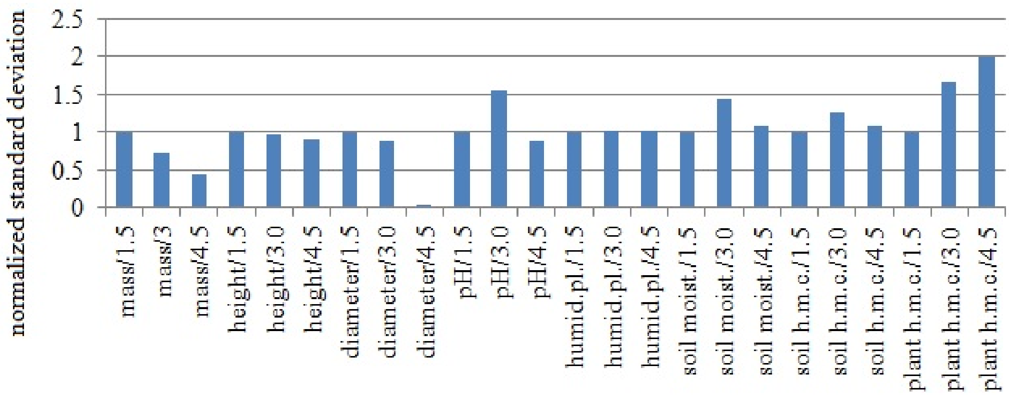


Figure 4. Variation of eight parameters of the plant after contaminating the soil, using the normalized standard deviation, in consideration of Zn accumulation (where humid.pl.: plant water content; soil moist.: soil moisture; soil h.m.c.: soil heavy metal concentration; plant h.m.c.: plant heavy metal concentration).

3. Results

3.1. Statistical Models (Polynomials Time Regression)

The statistical methods used to obtain the models are based on linear regressions (first-degree polynomials), quadratic regressions (second-degree polynomials), cubic regressions (third-degree polynomials), and the polynomials regression by fourth-degree poly.

Polynomial regressions, and non-polynomial regressions, are obtained by applying the least squares method. In the cases of the polynomial regressions, the sought coefficients are obtained by solving linear equations systems, coming from the calculation of the critical points. The calculations of the coefficients from the interpolation polynomials were made using the interpolation function of the MathCAD 15 program, whose algorithm is described in study [25]. In addition to the regression polynomial coefficients, the polyfitc function of the MathCAD 15 program also provides other important statistical features:

the standard error for the regression coefficient, the lower and upper boundary for the confidence interval of the regression coefficient, the variance inflation factor—the measure of the inflation of the regression coefficient due to multicollinearity, Student's t-test statistic if the term is significant, and *p*-value—probability of rejecting the term based on its t-statistic, when in fact it is significant. The terms used in statistical analysis are presented schematically in Table 1.

Table 1. Presentation of the main terms used in statistical analysis.

Term	Labels for Each Term Reported Upon
Coefficient	Regression coefficient for each term
Std Error	Standard error for the regression coefficient
95% CI low	Lower boundary for the confidence interval of the regression coefficient
95% CI High	Upper boundary for the confidence interval of the regression coefficient
VIF	Variance inflation factor: measure of the inflation of the regression coefficient due to multicollinearity
T	Student's t test statistic to test if the term is significant
P	*p*-value: probability of rejecting the term based on its t-statistic when in fact it is significant

As an example, Table 2 shows the final and intermediate results of the interpolation for the evolution of the mass of plants grown in soil with a zinc concentration of 4.5%. The significance and calculation algorithms of these characteristics were calculated according to [25] and the PTC MathCAD prime linear regression, (2018).

Table 2. Results of statistical analysis, for the interpolation (using first-degree polynomial) of lettuce mass variation over time, grown in soil with 4.5% zinc concentration.

Term	Coefficient	Std Error	95% CI Low	95% CI High	VIF	T	P
t^0	2.25	0.874	0.272	4.228	NaN	2.573	0.025
T	0.117	0.021	0.069	0.166	1	5.517	0.0002401

According to Tables 2–5, the equation that gives the time dependence of the plant mass of lettuces grown in soil with a concentration of 4.5%, through the polynomial function, are the following:

$$\begin{aligned} m1_{4.5}(t) &= 2.25 + 0.117t \\ m2_{4.5}(t) &= 1.656 + 0.175t - 0.0008428t^2 \\ m3_{4.5}(t) &= 0.59 + 0.424t - 0.01t^2 + 0.00009331t^3 \\ m4_{4.5}(t) &= 0.142 + 0.657t - 0.028t^2 + 0.0005041t^3 - 0.000003033t^4 \end{aligned} \tag{2}$$

where: t is the time (in days).

Table 3. Results of statistical analysis, for the interpolation (using second-degree polynomial) of lettuce diameter crowns variation over time, grown in soil with 4.5% zinc concentration.

Term	Coefficient	Std Error	95% CI Low	95% CI High	VIF	T	P
t^0	1.656	1.218	−1.153	4.466	NaN	1.359	0.152
t	0.175	0.083	−0.017	0.368	14.5	2.106	0.053
t^2	−0.0008428	0.001168	−0.003536	0.00185	14.5	−0.722	0.291

Table 4. Results of statistical analysis, for the interpolation (using third-degree polynomial) of lettuce diameter crowns variation over time, grown in soil with 4.5% zinc concentration.

Term	Coefficient	Std Error	95% CI Low	95% CI High	VIF	T	P
t^0	0.59	1.309	−2.506	3.685	NaN	0.45	0.343
t	0.424	0.176	0.008801	0.839	76.185	2.415	0.034
t^2	−0.01	0.006169	−0.025	0.004191	478.927	−1.685	0.099
t^3	0.00009331	0.00005934	−0.000047	0.0002336	200.478	1.573	0.115

Table 5. Results of statistical analysis, for the interpolation (using fourth-degree polynomial) of lettuce diameter crowns variation over time, grown in soil with 4.5% zinc concentration.

Term	Coefficient	Std Error	95% CI Low	95% CI High	VIF	T	P
t^0	0.142	1.427	−3.35	3.634	NaN	0.099	0.381
t	0.657	0.321	−0.129	1.444	246.875	2.044	0.06
t^2	−0.028	0.021	−0.079	0.023	5281	−1.331	0.155
t^3	0.0005041	0.0004744	−0.0006568	0.001665	12380	1.063	0.209
t^4	−0.000003033	0.000003474	−0.00001153	0.000005468	2948	−0.873	0.252

The interpolation curves corresponding to the polynomial Equation (2) are shown in Figure 5.

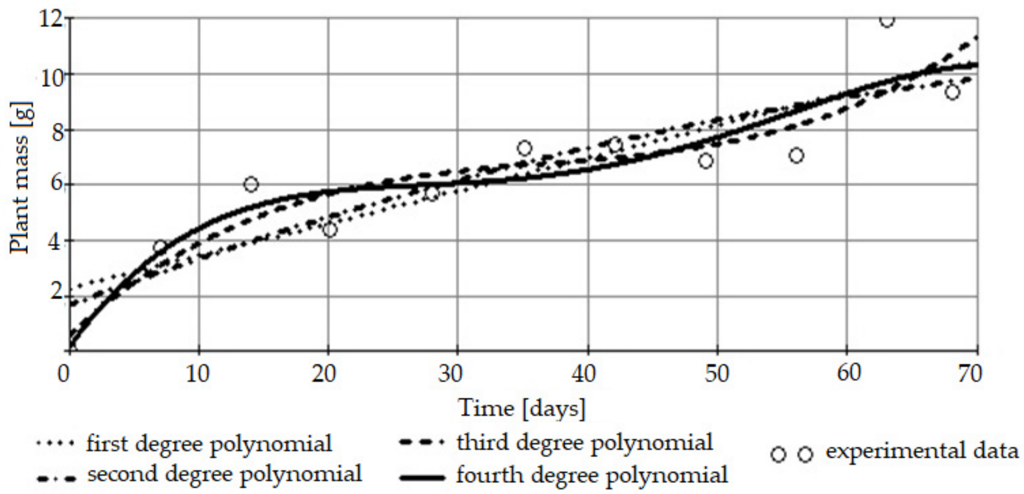


Figure 5. Time dependence of the mass of lettuces grown in soil contaminated with 4.5% zinc concentration, for 1 to 4degree polynomial interpolations.

Similarly, the polynomial regression expressions of time dependence were obtained for the other two soil contaminations, for the plant mass and their height, in accordance with the way lettuces have grown in the three types of soil. The graphical representations from Figure 6 demonstrate the regressions obtained.

The three characteristics represented graphically have a generally monotonous tendency (within the entire 68-day monitoring period). Polynomial curves of a higher degree show that there are some points of local extreme, which could deviate from reality, which should be further researched from a biological or physical perspective. It can be observed that the linear interpolation confirms the inverse proportionality between the intensity of soil contamination and the increase of the plant's mass. The second-degree interpolation curves show that towards the end of the monitoring period, the three characteristics (mass, Zn concentration, and height) cease increasing or even have decreasing tendencies. This behavior is most likely generated by the polynomial function of the second degree. However, in the case of plant mass, both the third- and fourth-degree interpolation functions indicate decreasing tendencies at the end of the monitoring period, but only for the lowest soil contamination concentration (1.5%). In the other cases, the tendency is increasing, more or less convincingly, until the end of the monitoring period.

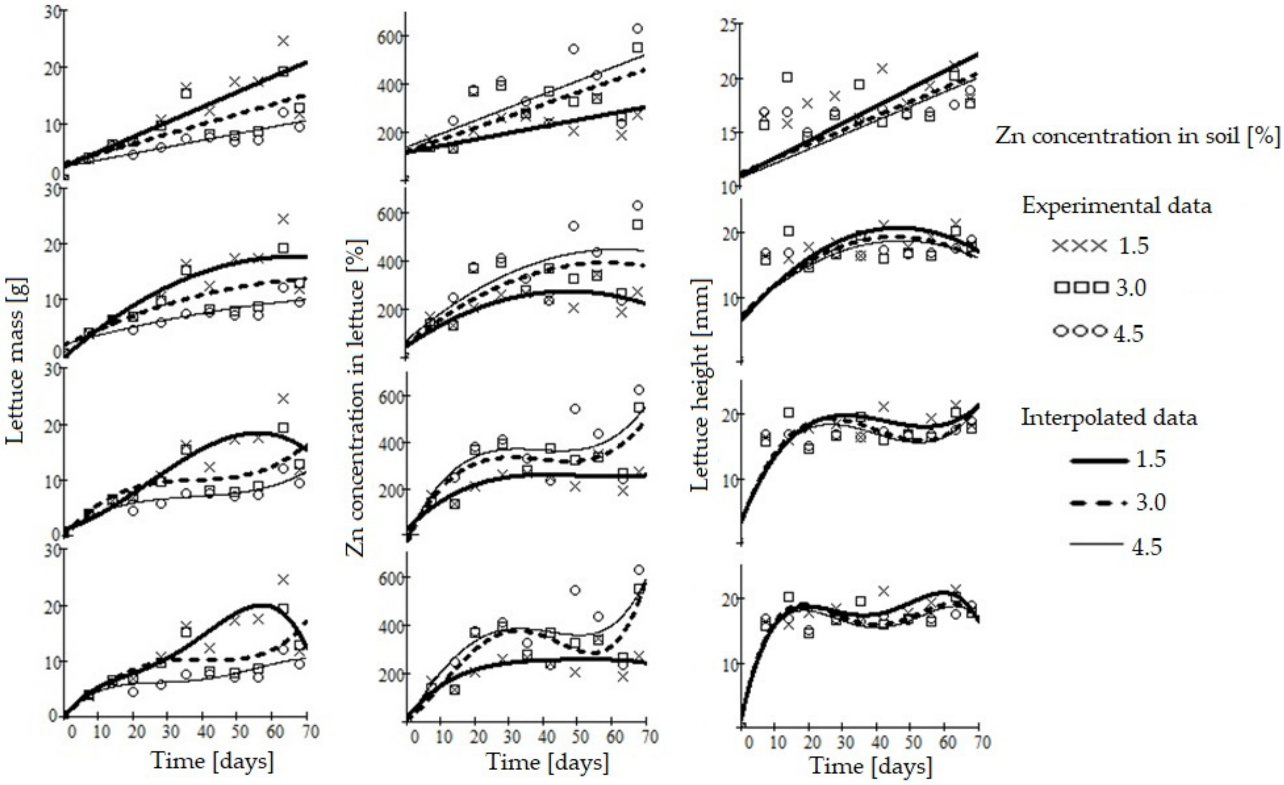


Figure 6. Graphical representation of Zn concentration, mass, and plant height over time, obtained by polynomial interpolation (1–4 degree ordered top to bottom of graph) and experimental data.

It is observed that the premises drawn for the linear interpolation case are also valid in this case (the monotony of the mass increasing and the inverse order reported to the level of Zn contamination of the soil). The parabolas depicted in Figure 6 present a maximum point (they all have the coefficient of the second-degree term negative). Theoretically, this means that there would be a time when the mass begins to fall. Since this maximum, for each of the parabolas, is outside the interpolation interval, this last statement remains subject to the validation of the extrapolation.

Starting with the interpolation by third-degree polynomials (Figure 6), the accuracy increases, the interpolation curves model increasingly better to the experimental data oscillations. In this context, this increases the challenge of explaining the origin or causes of the oscillations reported to a monotonic average curve or, relatively, to the linear regression curve (the straight line).

For example, in the case of the third- and fourth-degree interpolations (Figure 6), the maximum from the penultimate harvest, provided by the experimental data, increases in clarity.

3.2. The Variation Obtained for the Concentration of Heavy Metals in Plants

With the same interpolation tools, similar results can be obtained regarding the increase of Zn concentration in plants. In Figure 6, the graphs of linear interpolation of the increase of heavy metal concentration in plants are also given, at three degrees of soil contamination. The experimental data are also represented in the graph. It is observed that in the more intensely contaminated soils, the accumulation of heavy metal in plants is more intense and occurs faster (the inclination of the corresponding straight lines is more pronounced).

Figure 6 also shows the variations of Zn concentrations in lettuce grown in the soils' different Zn concentrations, in the interpolated form of grade II polynomials together with the experimental data. It can be easily observed that the same order of heavy metal concentrations is in the plants of the three categories of soil contamination, as in the case of the linear interpolation. Zinc accumulation in the plant is more intense, as the initial concentration of zinc in the soil was higher.

The order of the variation curves of heavy metal concentrations and their monotony increase in the plants is constant for the third-degree polynomial, as well as the separation of the curves (there are no intersections between the three curves). The third-degree polynomial interpolation curves, for the concentration increase in plants, are represented in Figure 6.

In general, the order and monotony of the interpolation curves of the variations of heavy metal concentration in plants are respected by the fourth-degree polynomial curves (Figure 6). With very few exceptions (the curve corresponding to soil contamination with 3.0% solution easily reaches the curve corresponding to 4.5% concentration and is very close, towards the end of the observation period, to the curve corresponding to plants grown on soil contaminated with 1.5% concentration solution), the separation of the curves is well respected.

3.3. *Measurement of Plant Height at Harvest*

Plant height, measured from the soil, at the time of harvest, is a significant growth parameter, and has been recorded for each plant at each stage of harvesting.

The linear interpolation leads to the regression curves in Figure 6. Plant height is a positive characteristic of evolution and, as expected, the behavior is similar to that of plant mass: the more intensely contaminated the soil is, the smaller the plant height at harvest.

3.4. *Statistical Models (Polynomials Time and Zinc Concentration in Plant Regression)*

Polynomial interpolations over time can be extended to the polynomial interpolations of two variables, where the second variable is the initial zinc concentration in the soil. The calculation method is similar to that used for polynomials that interpolate the temporal evolution of lettuce culture characteristics. Given the multitude of experimental data, interpolation by polynomials of two variables could be performed up to the third degree. This paper gives an example of the calculation of the interpolation polynomials of two variables of degree 1–3, for the plant mass. Similar interpolations are made for the evolution of plant height and diameter. They can also be made for any of the other characteristics measured during the experiments.

The result of the statistical interpolation calculation for the polynomials of two variables is given similarly to that for the polynomials of one variable, in Tables 6–8. The discussions made for this statistical calculation method are the same as those made for the polynomial interpolation with a single variable.

Table 6. Statistical characteristics of the linear regression by two variables for the mass of lettuces.

Term	Coefficient	Std Error	95% CI Low	95% CI High	VIF	T	P
$t^0, {c_{Zn}}^0$	4.402	2.889	−1.498	10.301	NaN	1.524	0.125
t	0.197	0.035	0.126	0.268	1.227	5.648	0.000005.271
c_{Zn}	−0.007352	0.009.692	−0.027	0.012	1.227	−0.759	0.295

The variation of plant biomass (lettuce) grown in zinc-contaminated soil is given in analytical form by Equation (3), for 1- 3-degree polynomials. Using Equation (3) and the experimental data, graphical representations of the time dependence of plant mass and the initial concentration in the soil were plotted. It is represented as portions of surfaces in three-dimensional space, the function of plant mass variation with time and the concentration of zinc, linear (Figure 7), square (Figure 8), and cubic (Figure 9), respectively. In each graph, the experimental data are represented by isolated symbols.

Table 7. Statistical characteristics of the cubic regression by two variables for the mass of the lettuces.

Term	Coefficient	Std Error	95% CI Low	95% CI High	VIF	T	P
$t^0, {c_{Zn}}^0$	3.221	11.917	−21.231	27.673	NaN	0.27	0.381
t	0.442	0.164	0.105	0.779	26.769	2.694	0.014
c_{Zn}	−0.021	0.073	−0.171	0.128	68.351	−0.293	0.378
$t \cdot c_{Zn}$	−0.0004303	0.000523	−0.001503	0.0006.428	44.753	−0.823	0.279
t^2	−0.00138	0.002.089	−0.005666	0.002905	22.054	−0.661	0.316
${c_{Zn}}^2$	0.00004.2	0.0001039	−0.0001713	0.0002553	59.502	0.404	0.363

Table 8. Statistical characteristics of the quadratic regression by two variables for the mass of the lettuces.

Term	Coefficient	Std Error	95% CI Low	95% CI High	VIF	T	P
$t^0, {c_{Zn}}^0$	−35.198	71.732	−183.586	113.191	NaN	−0.491	0.348
t	1.07	1.294	−1.606	3.746	1470	0.827	0.278
c_{Zn}	0.317	0.772	−1.281	1.914	6780	0.41	0.362
$t \cdot {c_{Zn}}^n$	−0.00343	0.00589	−0.016	0.00876	5010	−0.582	0.331
t^2	−0.0748	0.014	−0.037	0.022	896.299	−0.528	0.342
${c_{Zn}}^2$	−0.000914	0.00261	-6.31×10^{-3}	0.00448	33100	−0.351	0.37
$t_2 \cdot c_{Zn}$	0.000000617	0.0000654	-1.35×10^{-4}	0.000136	3020	0.009.44	0.395
$t \cdot {c_{Zn}}^2$	0.00000469	0.00000645	-8.66×10^{-6}	0.000018	1040	0.727	0.3
t^3	0.0000542	0.000170	-2.97×10^{-4}	0.0004.06	582.308	0.319	0.374
${c_{Zn}}^3$	0.000000853	0.00000268	−0.00000468	0.00000639	9500	0.319	0.374

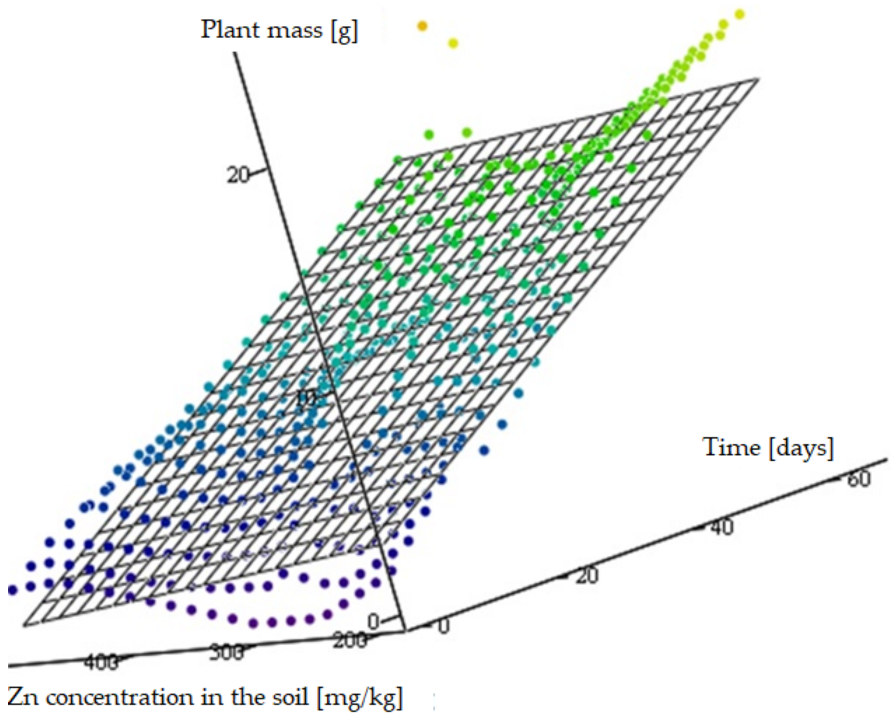


Figure 7. Variation of lettuce mass with time and the concentration of Zn in the soil, as a three-dimensional surface, corresponding to linear interpolation.

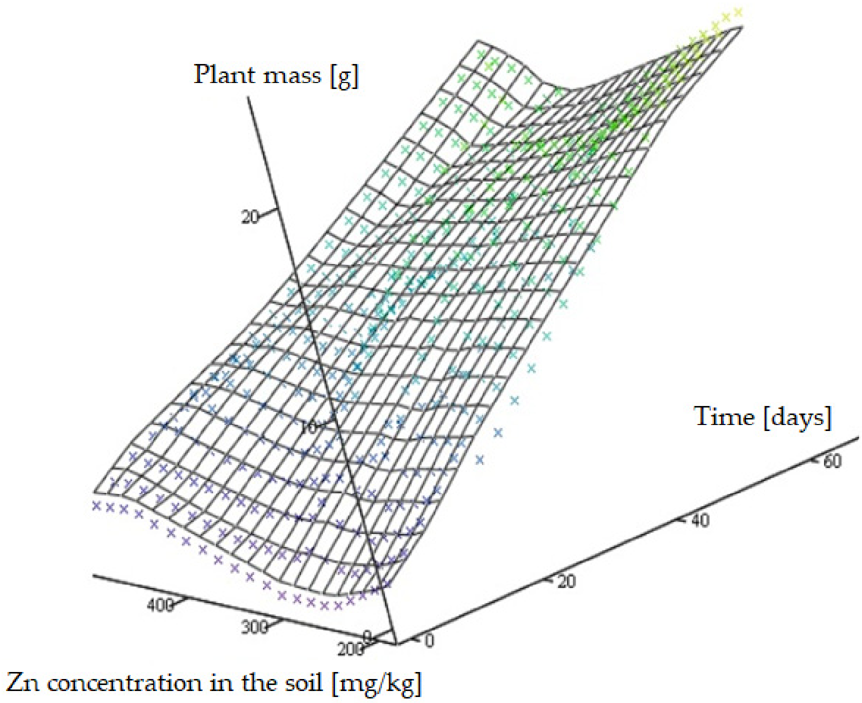


Figure 8. Variation of lettuce mass with time and the concentration of Zn in the soil, as a three-dimensional surface, corresponding to a second-degree polynomial interpolation.

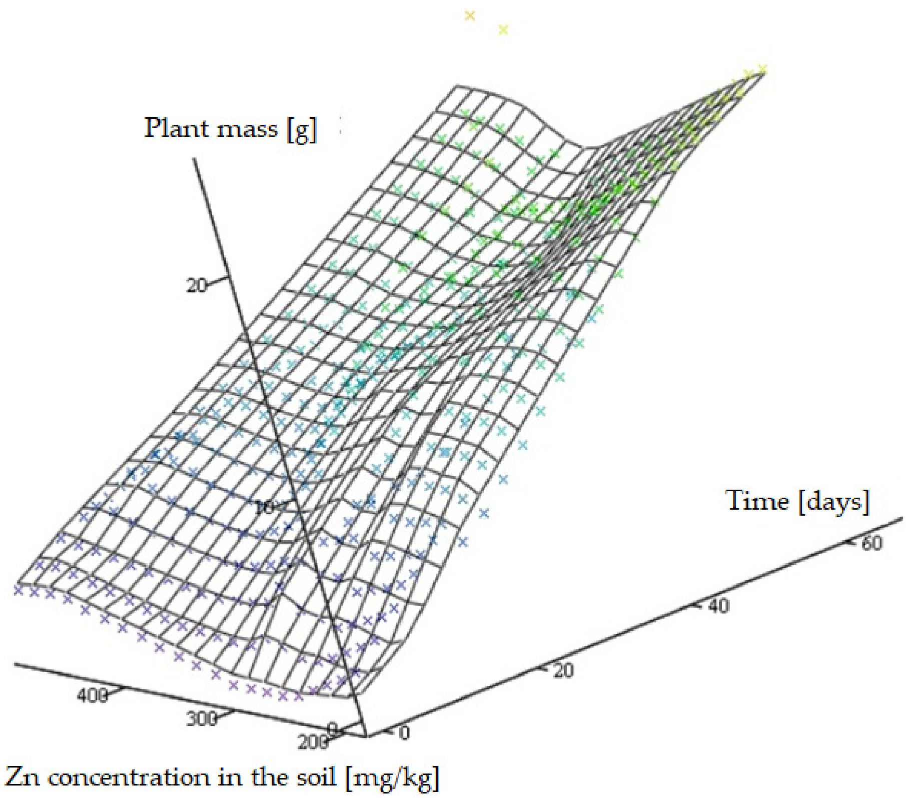


Figure 9. Variation of lettuce mass with time and the concentration of Zn in the soil, as a three-dimensional surface, corresponding to a third-degree polynomial interpolation.

The analytical expression of the three interpolation polynomials is obtained using Tables 6–8:

$$
\begin{aligned}
m(t, c_{Zn}) &= 4.402 + 0.197 \cdot t - 0.007352 \cdot c_{Zn}, \\
m(t, c_{Zn}) &= 3.221 + 0.442 \cdot t - 0.021 \cdot c_{Zn} - 0.0004303 \cdot t \cdot c_{Zn} - 0.00138 \cdot t^2 + 0.000042 \cdot c_{Zn}^2, \\
m(t, c_{Zn}) &= -35.198 + 1.07 \cdot t + 0.317 \cdot c_{Zn} - 0.003427t \cdot c_{Zn} - 0.007482 \cdot t^2 - 0.0009143 \cdot c_{Zn}^2 + \\
&\quad 0.000000617 \cdot t^2 \cdot c_{Zn} + 0.000004691 \cdot t \cdot c_{Zn}^2 + 0.00005423 \cdot t^3 + 0.0000008533 \cdot c_{Zn}^3
\end{aligned}
\tag{3}
$$

4. Discussion

The three areas of the surfaces drawn in Figures 7–9 show that the plant's mass increases over time; however, it tends to decrease when the initial concentration of zinc in the soil increases. This property is difficult to highlight, because:

- Zinc is also an important nutrient in plant development so at an early stage of development its rapid accumulation could promote biological growth;
- Some oscillations, which appeared in the evolution of the mass, height, or diameter of the plants, may appear due to some parameters that were not considered in the model (even if the crop was hosted in the greenhouse, lighting and temperature were greatly influenced by atmospheric conditions);
- There is a high possibility that at certain values of the accumulation of heavy metals, the plants at some point may adopt a mechanism of rejection of those elements, similar to a defense mechanism (this is a hypothesis resulting from the study and applicable and testable in deterministic models that can be built).

The variation over time of the mass increase of lettuces grown on soil contaminated by zinc (Figure 6) confirms the results of the theoretical prediction made in another study [26]. All the interpolations perform a mediation of the experimental data using the method of least squares. It can be noticed that the increase of the interpolation polynomial degree leads to a better approximation of the experimental data; however, we have no reasons of a phenomenological nature to increase the degree of polynomial interpolation over the third degree. Another reason why we are not tempted to increase the interpolation degree is that the functions found in this way are very particular and useful only in the experimental range reached for the working parameters, the extrapolation being contraindicated. For these reasons, the statistical model can be used to validate the theoretical (dynamic) model, which is generalizable not only in terms of climatic conditions, but also depends on the types of plants used.

A number of recent studies [14,27–29] are in agreement with the results we obtained and presented in this paper.

The results of the experimental data processing show that lettuces grown in soils with a higher Zn concentration have lower heights. This phenomenon is observed in many plants, for example, the authors in study [14] evaluated the presence of cadmium and zinc in sunflower and in the soil. This result is synthetically visible in the results shown in Figure 6, on the third column of the graph. It is observed that the regression curves corresponding to the plants grown in the soil with the highest concentration of Zn, are located below the regression curves corresponding to the plants grown in the soil with average zinc concentration. The regression curves corresponding to plants grown in medium contaminated soil are below the curves that represent the increase in height of the plants grown in low soil contamination.

Similar remarks on the development of the *Brassica junceea* are presented in study [27]. The influence of zinc, however, is manifested starting from a higher concentration threshold in the soil (500 ppm), that can be classified as very high. Table 4 shows the influence of increasing the concentration of zinc in the soil, that leads to a decrease in the height of the plants and a decrease in the mass. The decrease in lettuce mass caused by the increase in zinc concentration in the soil is synthetically represented in Figure 6, in the first column of the graph. The growth curve of the lettuce mass cultivated in the soil with the highest zinc concentration is located below the curve corresponding to the plants cultivated in the soil with medium zinc concentration, and is placed under the growth curve of the plant mass grown in the soil with the lowest concentration of zinc. Similar behavior to the height and mass assessment could be seen on the diameter of the crown of the plant.

The effects we noticed following the experiments performed on the lettuce are also confirmed by study [29] which mentions the effects of zinc concentration in the soil: reduction in height and biomass for cluster bean (*Cyamopsis tetragonoloba*), and development slowdown for pea (*Pisum sativum*) and rye grass (*Lolium perenne*). Another study [30,31] also concluded that the toxicity of zinc in plants is visible through the effect of the inhibition of growth and diminishes in biomass generation, while the high toxicity may be fatal. Table 9 validates the result obtained by statistical analysis study with other papers identified in the literature.

Table 9. Table summarizing key findings for the development of *Brassica junceea* in similar studies.

The Main Conclusion of the Analyzed Papers	Papers That Confirm the Conclusions of the Present Research
The mass of all plants increases over time, (with several small variations). It has not been determined what are the effects produced by high concentrations of heavy metal, with the emphasis on the doses at which the germination of the plant or its death could emerge	[14,27,29–31]
The mass of the plants at each stage of harvesting is inversely proportional to the initial concentration of zinc in the soil	[27,29–31]
The height of the plants harvested at the same time is inversely proportional to the initial concentration of zinc in the soil	[14,27,29–31]
The diameter of the plants harvested at the same time is inversely proportional to the initial concentration of zinc in the soil	[27,29–31]

All the interpolations made within the statistical models that appear in the present paper make mediation of the experimental data. It can be noticed that the increase of the interpolation polynomial degree leads to a better approximation of the experimental data; however, we have no reasons of a phenomenological nature to increase the degree of the interpolation polynomial over the value of three. Another reason why we are not tempted to increase the interpolation degree is that the functions found in this way are very particular and useful only in the experimental range reached for the working parameters, with the extrapolation not being indicated. For these reasons, the statistical model can be used to validate the theoretical (dynamic) model, which is generalizable not only in terms of climatic conditions but also for other types of plants.

The variation over time of the increase of lettuce mass grown on soils contaminated with zinc confirms the result of the theoretical prediction. More precisely, the increase of the pollutant concentration in the soil leads to a slower increase, and to the development of plant specimens with a smaller mass (also smaller height and diameter) than the plants from the same lot, grown on soil not contaminated with zinc. The plants are being studied in interaction with the soil and the growing conditions, throughout their lifetime (if we can define the lifespan, i.e., to specify the time of death of a plant). There are many more parameters that influence the development process of plants affected by excessive heavy metal content. Atmospheric parameters that play an important role in plant development (temperature, moisture, lighting, etc.), and parameters of soil structure and composition (compactness, density, porosity, nutrient content, and chemical composition in general), must be considered in experiments. Due to a large number of influential parameters, the number of experiences will increase in consequence. For this reason, wide international collaborations are recommended to carry out such experiments on portions distributed to various laboratories, under centralized monitoring.

5. Conclusions

Correlating plants' growth characteristics with the soil contamination levels using statistical modeling can obtain interpolated variations of the mass increase functions to determine several functions that express the time dependence of the heavy metal concentration in the lettuce plants. Starting from these interpolation relations, one can calculate other characteristics of the evolution process for various plants that are growing on contaminated soils.

We identified a research tool that describes how different levels of zinc contamination affects lettuce crops, analyzing three growth characteristics: plant mass, plant height, and plant diameter. We used the least squares method on the experimental data, in order to determine the 1–4 grades mathematical regression functions that describe the three growth characteristics in time. The three plant growth characteristics (mass, height, and diameter) have been correlated with the three zinc concentrations in the soil in order to draw conclusions about the effect associated to each concentration on the lettuce experimental culture.

The main conclusion of the statistical model, that was validated by experimental determinations, is the appearance of the dwarfism effect. Therefore, as the concentration of zinc in the soil increases, lettuce plants tend to have a smaller size and mass. The functions that describe the mass, height, and diameter, and variation over time, have been extended for two variables: time and zinc concentration. In this way, a better prediction was obtained regarding the plant development, under the influence of zinc concentration in the soil. All these functions can then be used for forecasting, using extrapolation.

The techniques that have been used are not necessarily new; however, they are very useful for forecasting and for studying the biodynamics of plant development.

The results obtained by the interpolation of the experimental data and their interpretation are likely to give very important indications for the following experimental step: the prolongation of the experiments over at least two to three generations of plants, which will allow drawing some conclusions on the transmission of heavy metals between generations of plants, or, probably depending on other influential parameters of the process, the decrease of the heavy metal concentration in the plants of the following generations.

Another important observation is the dependence of the contamination level on the type of soil used. Zinc contamination tests should target several soil types, given the different way in which soil structure can affect the time behavior of pollutants.

The paper addressed a specific situation where the contamination was achieved by applying a fixed dose of zinc, while the synergistic effects with other substances were not evaluated. It should be noted that the contamination process found in nature may be different: the contamination might be either continuous for long periods of time, or intermittent with maximum and minimum values.

Our experiments will continue the study with the evaluation of plant development for several other soil types, with the analysis of the synergistic effects between pollutants, and with the variation of the concentrations used for soil contamination.

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