PROJECT PRESENTATION

„RESEARCH ON THE DEVELOPMENT OF SOME MATHEMATICAL MODELS TO EVALUATE THE IMPACT OF SOIL CONTAMINATION ON FRUITS AND VEGETABLES”

Project code: PN-III-P4-ID-PCE-2016-0860
The acronym of the project: CONTAMOD
Implementation period: 01/2017 - 12/2019

OBJECTIVE OF THE PROJECT

To achieve the main objective of the project, *the development of some mathematical models to evaluate the impact of soil contamination on fruits and vegetables* to be consumed by the population in large scale, the following ***specific objectives*** are aimed:

O1) Scientific substantiation on the models used worldwide to evaluate the contamination of soil, respectively fruits and vegetables;

O2) Development of theoretical models to evaluate the impact of soil contamination (by heavy metals and other pollutants) on fruits and vegetables. Establishing of work assumptions;

O3) Testing of theoretical models in real conditions based on data obtained from laboratory / field;

O4) Development of mathematical models to evaluate the impact on soil contamination on fruits and vegetables and thus on consumers health;

O5) Wide dissemination of the results and development of proposals for Ph.D themes.

SUMMARY OF THE PROJECT

Environmental pollution is a major problem worldwide, and in this context the European Union adopted in the past 20 years numerous laws, directives and standards for its mitigation, monitoring and if possible, its control.

Soil pollution is a component that contributes to the increase of environmental pollution, as soil is the key element that underpins food production for household consumption. If we refer only to the soil, the level of pollution is given by its degree of contamination with various pollutants, heavy metals, etc.

Because agricultural products in general, respectively fruits and vegetables in this case extract water and nutrients from the soil in order to develop, it is necessary to study how a certain degree of soil contamination (at different depths) leads to the obtaining of some products (vegetables and fruits) which contain a percentage of those toxic elements, that can produce long term sickness and can cause death in people who currently consume such contaminated products.

The research carried out within the project aim to develop some original models *on the correlation between the level of soil contamination, respectively the remanence of polluting substances in fruits and vegetables harvested* for consumption in fresh state and of an optimal method to *reduce / improve / remove / dismantle / control the pollutant substances from the soil*, which will be correlated with a monitoring system of activity and impact of polluting substances / elements in a certain area for rapid improvement actions with neutralizing substances.

PHASES / ACTIVITIES:

Den. No.	Phase / activity name	Objectives	Deadline
P1	**Scientific substantiation of mathematical models used worldwide to assess the impact of soil contamination on fruits and vegetables**	• Scientific substantiation of models used worldwide to assess soil contamination, respectively fruits and vegetables contamination; • Development of theoretical models for assessing the impact of soil contamination (with heavy metals and other pollutants) on fruits and vegetables. Establishment of working hypotheses	**12.2017**
A1.1	Prospective study on mathematical models in the field of soil contamination impact on fruits and vegetables		
A1.2	Establishment of the experimentation methodology		
P2	**Experiments in the laboratory / field. Development of mathematical models**	• Experimentation of theoretical models under real conditions based on laboratory / field data	**12.2018**
A2.1	Experiments under laboratory / field conditions		
A2.2	Development of mathematical models		
P3	**Development of a method for improving parameters to ameliorate soil contamination**	• Development of mathematical models for assessing the impact of soil contamination on fruits and vegetables and, implicitly, on consumer health; • Wide dissemination of results and elaboration of proposals of PhD thesis topics	**12.2019**
P3.1	Experiments in the laboratory / field		
P3.2	Validation of mathematical models		

Results / results presentation documents:

- 7 ISI articles;
- 13 BDI articles;
- 1 European patent application.

Degree of achievement of estimated results:

PHASE 1 - *SCIENTIFIC SUBSTANTIATION OF MATHEMATICAL MODELS USED WORLDWIDE TO ASSESS THE IMPACT OF SOIL CONTAMINATION ON FRUITS AND VEGETABLES*

Activity 1.1. – Prospective study on mathematical models in the field of soil contamination impact on fruits and vegetables

According to [1], some of the substances that form the crust of the Earth are elements, substances that cannot be decomposed into simpler substances. Some of these elements are poisonous, even if they are present in a low concentration. These elements are known as heavy metals. Among the heavy metals, [1] are mercury, cadmium, arsenic, chromium, thallium and lead.

Bioaccumulation [2] is defined as the accumulation of substances (e.g. pesticides) in organisms of various types. Also [2] states that ***bioaccumulation*** occurs in organisms when *absorption* takes place at a faster rate than the *elimination* of the same substances by *catabolism* or *excretion*.

According to [6], *bioconcentration* is a term related to *bioaccumulation*, but more specifically, it refers to the accumulation of a substance only from water. By contrast, bioaccumulation refers to combined absorption from all sources (water, food, air, etc.), [7]. However, the term bioaccumulation has a beneficial sense, as long as it is not associated with substances harmful for certain biological or physical entities. According to [8] or [10], *bioaccumulation* is a process of accumulation, in the soil, of organic substances resulting from the decomposition of vegetal and animal debris that contributes to soil fertilization. [8] also calls *bioaccumulation* the process of accumulation, in the plant, of some substances, which, after decomposition of the plant material, remain in the soil, fertilizing it. Therefore, bioaccumulation is a process of accumulation of substances in a biological entity (soil, plant, animal, etc.). The restrictive meaning of some definitions of *bioaccumulation*, relative to substances that are toxic for living organisms (pesticides, heavy metals, etc.), for example [9], is not recommended because the term may extend to the accumulation of other useful substances and recommends it for studies on living organisms' development within mathematical models.

The mathematical models of heavy metal bioaccumulation are part of the general category of mathematical models describing phenomena in the field of biology. These models are included in that branch of biology called biomathematics, [16] which is the branch of biology that deals with the application of mathematical principles in biology and medicine. Biomathematics has many applications in well-known branches of biology: Comparative Genetics, Population Genetics, Neurobiology, Cytology, Pharmacokinetics, Epidemiology, Oncology, or Biomedicine.

As stated by [18], the progress of scientific understanding in the field of biology and ecology is dependent on the accumulation of factual data, the creation of theories capable of structuring data and explaining phenomena, descriptive modeling of biological realities, and analysis of the findings validity. Although the phenomena of the living (biological) world are based on physicochemical phenomena, says [18], biological phenomena are incomparably more complex than physicochemical phenomena.

[37] states that the classification of mathematical models in biology is heterogeneous, a situation generated by the many possible points of view. The authors [39] give the following classifications:

C1) ***physical*** models and ***abstract*** models;
C2) ***dynamic*** models and ***static*** models;
C3) ***empirical*** models and ***mechanistic*** models;
C4) ***deterministic*** models and ***stochastic*** models;
C5) ***simulation*** models and ***analytical*** models;

Physical models are material models made on a scale that behave similarly to the modelled system. *Abstract* models are built in the human spiritual space and are constructed by systems, components and relations that imitate the functioning of the studied process. *Dynamic* systems are those in which the temporal variable is explicit (simulators based on systems of differential equations and/or partial derivatives). A *static* model is described by parameters and relations in which time does not appear explicitly (for example, regression models such as Fibonacci sequence to simulate changes in the number of rabbit populations). *Empirical* (correlation) models contain empirical relations, do not exhaustively observe the representation of the processes and mechanisms that take place in the real process, and their purpose is to predict and not to explain the causal relations in the phenomenon. *Mechanistic* models aim primarily at describing the internal dynamics of the system and determining the causality of its behaviour by observing the characteristics of its own real system, [37]. *Deterministic* models are characterized by the absence of random variables and lead, under identical initial and loading conditions, to identical predictions. *Stochastic* models include random variables and are more complex than the deterministic ones because, apart from the need to determine constants, it is necessary to determine complete distributions of values assigned to random variables. Generally, by *analytical* methods, [37] understands all methods that are solved using mathematical methods and give analytical solutions. The use of numerical methods or other methods of solving in the model give a *simulative* character to the model, [37].

Bioaccumulation modeling has long spread in the literature on ecological risk estimation [52]. There are a series of studies on the subject of bioaccumulation in invertebrate and small vertebrate species and use statistical models for this purpose [53]. The study of the bioaccumulation phenomenon in sediments or plants appears also in [54] and [55].

In [51], the authors propose a way of evaluating models dedicated to bioaccumulation processes:

- identifying chemicals of interest,
- selecting the factors that influence bioaccumulation potential variation,
- developing the models,
- verifying the viability of the models (validation),
- selecting the best predictions,
- making qualitative and quantitative adjustments,
- selecting key/main predictions or prediction domains and including them for the relevance of the bioaccumulation model.

We can recognize in this way the method of general modeling of processes as systems. In contrast to classical systems in mechanics or those in other classical fields of the art (electromagnetism, thermodynamics, etc.), ecological systems are generally open systems, or systems that cover spatial domains but also temporal domains of very large dimensions. These are specific characteristics of these systems and implicitly the great variability in time and space.

The current trend in modeling bioaccumulation phenomena is to achieve mixed models, which have deterministic components and statistical components. These models are called integrated models. Integrated modeling appears to be absolutely necessary for predicting the transfer of the stable toxic pollutant in a trophic chain that includes organisms characterized by multiple space-time scales. An example of integrated model can be found in [56], and a synthesis of the potential and limits of integrated models in metal biogeochemistry is found in [45].

We further mention some bioaccumulation models for possible consultation when it comes to constructing a mathematical model for bioaccumulation to model a particular phenomenon, concretely. The consultation of these models may tell the researchers if they are on a path already investigated or how far they have moved away from it.

Among the predictive deterministic models (which are difficult to parameterize as they have a complex structure), there are, [37]:

- Simulation of metal bioaccumulation in conifer and deciduous seedlings, using a 32-parameter model describing the concentration of metals in soil and air, metal properties and metal exposure time, take-up rates in different plant tissues and transfer between tissues, metal partition coefficients between the compartments of soil-plant system and aqueous phases, plant growth and metabolism parameters, [61];
- Simulation of metals transport by percolation and soil and their taking over by the plants, [62] - the detailed part from the point of view of the deterministic modeling is at the level of the water transport,

the taking over by the plants being very simply modelled by a Michaelis – Mentin equation. The model includes 24 parameters of which 6 characterize the plants.

- Deterministic models of the bioaccumulation of metals are also presented in [47], [49] and [49]. These models simulate the total transport of metals in the plant. The specific application of the model is the simulation of metals phytoextraction from the soil, [49], the simulation results being also relevant for assessing the dynamics of the bioaccumulation process.

Generally, in respect to the deterministic models it can be stated that besides the lack of knowledge about their correct structure for bioaccumulation prediction, an important restriction is the availability of data for parameterization and verification of such models in different types of terrestrial ecosystems, [63].

Statistical models are used to obtain, analyse and interpret data. Statistical models establish relations between variables, without considering the internal mechanisms of processes and without explaining their causality, [64]. The author [65] considers that the statistical models have three important functions:

- They can be tested and verified by real data,
- They can be used to analyse the real data quality when considered abstract and used to describe real phenomena,
- When used for parameter estimation they can suggest emerging properties of the systems or characterize the dynamics of processes and may play a role in resource management.

Also [37] shows that regression models can often be used to determine the form and significance of the relation between two or more variables. We add that, among a certain family of forms one can find, quite often an optimal one, using the method of least squares. Another very tenacious and helpful "assistant" of these operations is dimensional analysis. [66] distinguishes between regressions and correlations: correlations measure the degree of linkage between variables, while regressions also reflect the intensity of the link, but these presuppose a causal relation between variables (one dependent and one or more independent).

In order to choose a model type in case we have to study a concrete process, the authors [40] propose a tabular guide, presented in table 7.

Table 7 - Guide to choosing a mathematical model

Model type	Characteristics	Selection criteria
Matrix representations	linear relations	valid linear equations, age structure required
Static models	provide an overview in quantitative terms of the situation	applied in situations where there is little data available and quantitative assessments are required while changes correlated with long time intervals are not required
Fuzzy models	provide semi-quantitative results or just indications on rankings	applied in situations where the available data are few and the semi-quantitative results are sufficient
Representations by differential equations	provide space-time variations	require a developed database
Models describing structural dynamics	provide parameter variations as a function of time and/or space based on expert knowledge or purpose of functions	predictions are required in the context of changing conditions and a database developed with changes in properties
Individual-based models	consider different properties of individualities	used where the average of properties/parameters is insufficient

Statistical models for predicting metal concentrations in plants have as main independent variables metal concentrations in the soil. Based on the available analytical data, the concentrations are total or only the bioavailable portion.

The experimental consequences of the mathematical modeling of heavy metal bioaccumulation phenomenon are immediate in the applications for the elaboration of rigorous experimental plans. Thus, the mathematical model and the problems proposed to be solved determine precisely all the parameters to be determined experimentally, as well as the locations in the analyzed system of these measurements (in which component of the system and in which subcomponent: for example in the plant, in leaves, stem, seeds, root, etc.). The complexity of the theoretical-experimental research, in a field where research cannot be conceived without experiment, becomes controllable, and the degree of depth results easily, using estimators resulting from the theoretical-experimental analysis process. Primary experiences will be used both to substantiate the model and validate it. When talking about model substantiation, in the experimental context, we refer to the experimental determination of the model constants. At primary validation, all experimental data will be the subject of experimental validation of the model. The final validation is done on complex loading cases and also partially determines the model limits.

The experimental plan generated by the biodynamic model has as model parameters the rates of contaminant taking over from the environment, k^u and the rate of contaminant elimination in the environment, k^e. Also, the unknown and dependent on contaminant type for the same soil-plant couple is the equilibrium concentration, c_w. Since, ex hypothesi, these two parameters are believed to be constant over time and under established experimental conditions (temperature, pressure, concentration limits, etc.), their value can be determined experimentally from a discharge experiment. Prepare a number of n_{rep} x n_t pots with one plant and the soil contaminated at a known initial value, c_0. The set of t indices of the n_t number represents the times at which n_{rep} pots will be sacrificed for the measurements. Consider $\{t_i/i=0,...,N\}$ the sequence of these moments. At each t_i moment n_{rep} pots are sacrificed and the concentration in the plant is measured, as well as the concentration in the soil, considered to be the concentration of the environment. The resultant range of measurements is: $\{(t_i, c_i, cs_i)/i=0,...,N\}$, where cs_i is the concentration of the contaminant in the soil (average value). Then, using the approximation of the derivative by finite differences, we can write the following N-1 equations:

$$(t_{i+1}-t_i)k^u c_w-(t_{i+1}-t_i)k^e c_i=c_{i+1}-c_i\,. \tag{18}$$

From this set of N-1 equations is formed a number of C_{N-1}^3 systems of three nonlinear equations. These nonlinear systems are solved (if possible), obtaining C_{N-1}^3 solutions:: $(k_j^u, k_j^e, c_{w,j})$, $j=1,...,$ C_{N-1}^3. The final solution can be obtained either through mediation or by a statistical study of the distribution of each component of the nonlinear systems solution. For each measurement at the t_i time, n_{rep} soil-potted plant systems are sacrificed, and each triplet of each measurement will have the second and third components (c_i and cs_i) calculated as arithmetic averages of those n_{rep} sacrificed and measured systems. Thus, besides the n_{rep}xN soil - plant systems isolated in separate pots, there must be considered a number of at least 3N control plants sacrificed at each t_i time, three of them, which are control systems, without contaminants. Using the control system, it is possible to compare the physiological evolutions of the contaminated plants with the healthy ones. This results in a number N=(n_{rep}+3)N of plant-soil systems in separate pots to be cultivated for experiments. Harvesting (sacrification) times will be established throughout the life of a plant, possibly in a season, in order to harvest the seeds too, so that one could evaluate the presence of the contaminant in the germs of the next generation of plants, eventually on those plants afterwards, the ability to take over other contaminant loads (higher or lower than the parents).

Conclusions

- It is noted, within the limits of the relevant literature consulted, that the models most often used in the study of heavy metals bioaccumulation are the deterministic and the statistical ones.

- Deterministic models are more complete from the theoretical standpoint, cover more parameters, provide causal relations and have a wide range of applicability. The limitations are due to the large number of parameters considered and to the related model constants, which requires a large amount of experimental determinations to identify these coefficients. Costs are not negligible because analyses are as complex as those in the case of statistical models, and, moreover, some of the coefficients are obtained from data by additional calculations, so not directly from analyses. Higher quality experiences require adequate statistical processing.

- Statistical models, in relation to deterministic ones, show a low level of complexity, describing exclusively the experimental conditions for which they were generated (extrapolation is generally risky). Statistical models can be used efficiently in the context of the unavailability of a sufficiently developed data set as well as instruments for monitoring and assisting the decisions associated with risk assessment in certain contaminated areas. The disadvantages of these models are represented by the lack of general character and the inability to provide causal relations for the studied processes.

- Obviously, there is in this case too, as for many other issues, a middle way, namely the use of integrated methods, including both deterministic and statistical models.

- Regarding the existing and public experimental data in Romania, [37] states that the availability is reduced to several series of data characterized by general information (average values, areas of variation, dispersions). With these resources it is impossible to generate statistical models of metals bioaccumulation in crop plants. It is also noted the lack of data georeferencing for the heavy metals pollution problem. As a consequence, it is impossible to properly assess the risk associated with a precise spatial delimitation within agro-systems. In fact, the author [37] notes the large deficiencies of the statistical models built for the Copsa Mica area, especially with regard to the delimitation of areas at risk of exceeding the alert threshold. The results are inconclusive in the correct assessment of the distance from the source at which there is the risk of exceeding the threshold.

- The assessment of the experimental efforts needed to achieve a model of transfer of contaminants containing heavy metals from soil to plants (either a deterministic or a statistical model) shows that the operation is very costly.

- Generally, due to the large volume of literature on the transfer of heavy metals from the marine or terrestrial environment to plants or animals, it is difficult to reach a reasonable threshold of originality. There are some problems that can be studied and can guarantee original and useful solutions, among which:

- *researching the existence of bioaccumulation capacity in plants (in animals or humans), the topic being also related to the threshold of intoxication or illness symptoms occurrence;*
- *researching the mechanisms that can lead to the death of plants (animals or humans) through heavy metal poisoning (and generalization to other chemicals);*
- *the way of transmitting the heavy metal deposit from infected parents to the offsprings, the phytoremediation capacity of infested offsprings;*

Activity 1.2. – Establishment of the experimentation methodology

The activity has as main objective the development of theoretical models for assessing the impact of soil contamination with heavy metals and other pollutants on fruits and vegetables. Establishment of working hypotheses

Through the specific objectives it is desired to carry out some complex researches leading to the development of original models regarding the correlation between the level of soil contamination and the remanence of the polluting substances in the fruits ***(apple, plum, sour cherry, raspberry, strawberry, blueberry, etc.)*** and vegetables ***(spinach, parsley, tomatoes, cucumbers, radishes, etc.)*** harvested for fresh consumption.

When soil conditions allow heavy metals to pass into the soil solution, the increased content of heavy metals in the soil presents a direct risk of soil pollution, of the plants that absorb it, of the humans and animals consuming the respective plants. In addition, heavy metals can be leached into groundwater or surface water and from there to affect humans and animals through drinking water.

The risk of soil and plant pollution depends on:

- plant species,
- chemical form of chemical elements in the soil,
- the presence of other elements, especially those that counteract the effect of metals and substances that counteract the absorption and desorption processes,
- the amount available in soil and soil and climate conditions.

The harmful effects of heavy metals depend on their mobility, their solubility in the soil. Therefore, in the case of soils polluted with heavy metals, the first improvement measures will aim at creating the conditions for the passage of heavy metals from the soil solution in stable forms. In any species, heavy metal concentrations may vary between different parts and organs of the plant, but also with the age of the plant. There are species that have the ability to concentrate at the level of different vegetative organs high concentrations of heavy metals. Therefore, in the polluted areas it is contraindicated the consumption of green vegetables, heavy metals reaching them especially through foliar absorption.

Soil sampling will be made from the two surface horizons because they are considered to be affected by pollution.

Treating soil samples taken for analysis is done according to SR ISO 11464/1998 - Soil Quality. Pre-treatment of samples for physico-chemical analysis. Thus, the samples will be dried in the oven and then ground with a soil electric mill.

Heavy metals to be analyzed: ***Pb, Cu, Zn, etc.***, according to the standard SR ISO 11047/1999 - Soil quality. Determination of cadmium, chromium, cobalt, copper, lead, magnesium, nickel and zinc from soil extracts by flame atomic absorption spectrometry. Metal extraction is done with concentrated sulphuric acid and 50% hydrogen peroxide using a Digestal HACH digestion apparatus.

Copper has a normal content in the soil of 20 mg/kg, an alert threshold for sensitive uses of 100 mg/kg and an intervention threshold of 200 mg/kg.

The determination of pH is made according to SR ISO 10390/1999 - Soil quality. Determination of pH using a pH meter with combination electrode.

<u>The biological material</u> is represented by plants the selection of which was made taking into account criteria such as: frequency of consumption, taxonomy (to represent different families), exposure conditions (surface to volume ratio, growth period), the part of the plant to be consumed (fruits, leaves, etc.), tolerance to diseases and pests and spreading.

For the controlled contamination experiment will be used: *vegetables* **- *lettuce (Lactuca sativa L. var.capitata), spinach (Spinacia oleracea), parsley (Petroselinum spp.)*** cultivated as leafy vegetables, **carrot (*Daucus carota*)**, **European radish** (***Raphanus sativus convar sativus***) cultivated as root vegetables, **cucumber (*Cucumis sativus*)** and **tomato** (***Solanum lycopersicum***) and *fruits* ***(apple – Malus spp.; plum – Prunus domestica; sour cherry - Prunus cerasus),*** but also *berries* (**strawberries** - ***Fragaria spp.; blueberries - Vaccinium myrtillus; raspberries - Rubus idaeus; blackcurrants – Ribes nigrum***) because:

- they are some of the most consumed vegetables and fruits, rich in nutrients;
- they are usually consumed as such, raw;
- among vegetables, they have the highest accumulation capacity of heavy metals, without the manifestation of phytotoxicity visible symptoms;

- the selected vegetables have a short life cycle and develop well under controlled environment conditions (greenhouses, solariums). The locations with the experimental plots are to be established later (greenhouse and field).

Working methods and equipment used:

- *Atomic absorption spectrophotometry*
- *Inductively coupled plasma optical emission spectrophotometry*
- *High-performance liquid chromatography*

Parameters of the soil-plant system in laboratory experiments (tab.1) consider:

- establishing working hypotheses to facilitate the development of experiments for building and applying mathematical models of the soil-plant system;
- the experiments to be carried out will be opened in the sense that parameters can be added or removed depending on the results obtained during their deployment;
- the source of calculating the number of experiments taking into account the life cycle of plants within the experiments;
- starting with 2 kinds of crops: vegetables from the group of leafy vegetables (lettuce, spinach, parsley, etc.) and then with fruit trees (apple, plum, etc.).

Table 1 - Parameters of the soil-plant system in laboratory experiments

No.	Parameter name	Notation	Unit of measurement	Number of variants
1	Plant type	P_i	-	***Vegetables -*** lettuce, spinach, parsley ***Fruits*** – apple, plum
2	Contaminant type	C_i	-	**Pb, Cu, Zn**
3	Crop type	Tc_i	-	2
4	Soil type			
4	The amount of soil in the pots / soil area allocated to field experiments	V_{ssa}	m^3	-
5	The function of concentration variation of the contaminant in the soil	c_s	-	-
6	The function of concentration variation of the contaminant in the plant root	c_{cr}	-	-
7	The function of concentration variation of the contaminant in the plant stem	c_{ct}	-	-
8	The function of concentration variation of the contaminant in the plant leaves	c_{cf}	-	-
9	The function of concentration variation of the contaminant in the plant seeds	c_{cs}	-	-
10	Number of measurements per plant life cycle	n_{mcv}	-	-

Types of contaminant loads (Pb, Cu, Zn)

Contaminant (Pb, Cu, Zn) loads, by their mode of administration, can be done in two ways:

- loading directly into the soil with contaminant solution on controlled depth, in relation to the seedling planting depth (the ratio between the application depth and the planting depth/the length of the seedling root can take an infinite number of values)
- contaminant loading by rain;
- air loading with contaminant content, but it is difficult to control it and there is the risk of environmental pollution.

In terms of time evolution, contaminant loads can be of the following types:

- zero load – characteristic of the control soil-plant system, free of contaminant (mandatory load in order to achieve the reference)

- initial load - non-zero at the initial moment, mandatory, which models a contamination isolated over time;
- periodic load **-** which models periodic loads (produced by industrial sources or other sources);
- progressive load over the entire life-cycle – monitors the eventual decrease of the plant life time and death during its vegetation period;
- other forms of load required by experiments deployment.

ARTICLES PUBLISHED IN 2017:

1. Despina-Maria Bordean, Vladut Nicolae Valentin, Ioan Caba, Ioan Gogoasa, Liana Alda , Camelia Moldovan, Diana Moigradean, Luminita Pirvulescu, *Mathematical and chemometrical models – tools to evaluate heavy metals contamination,* Scientific Papers: Animal Science and Biotechnologies, 2017, 50 (2), pp. 162-167, ISSN online 2344 – 4576.

2. Despina-Maria Bordean, Nicolae-Valentin Vladut, Ioan Caba, Luminita Pirvulescu, Diana Nicoleta Raba, *Magnesium - an ancient mineral and the today's deficiency,* PROCEEDINGS OF THE 18th International Multidisciplinary Scientific GeoConference SGEM 2-8 july 2018, volume 18, Issue: 5.1, Ecology, Economics, Education and Legislation, Albena, Bulgaria, ISSN 1314-2704.

3. Pruteanu A., Bordean D.M., Voicea I., Vlăduț V., Găgeanu I., *Experimental researches concerning heavy metals contamination (Cu, Zn, Pb) in leafy vegetables,* PROCEEDINGS OF THE 18th International Multidisciplinary Scientific GeoConference SGEM 2-8 july 2018, volume 18, Ecology, Issue: 5.1, Economics, Education and Legislation, Albena, Bulgaria, pp. 385-392, (DOI:10.5593/sgem2018/5.1).

4. Petru Cardei, Catalina Tudora, *Theoretical research on evolution of health of plants affected by heavy metal absorption process***,** Engineering for Rural Development, Jelgava, 23.-25.05.2018, pp. 893-897, DOI: 10.22616/ERDev2018.17.N186.

POSTERS:

1. Pruteanu A., Vlăduț V., Nițu M., Voicea I., Bordean D., *Contaminarea controlata a solului cu metale grele si remanenta acestora in spanac*, 7th International Conference on Thermal Equipment, Renewable Energy and Rural Development 2018 TE-RE-RD.

Phase II – LABORATORY / FIELD EXPERIMENTS. DEVELOPMENT OF MATHEMATICAL MODELS

Activity 2.1. – Experiments in laboratory / field conditions

Growing of vegetables in greenhouses and solars. Growing of bushes and fruit trees

In this chapter, a documentation was made regarding the cultivation of vegetables [1,2,3,4,5,6,7,8] and fruits [12,13].

Development of experimental research

Choosing of plants for the study

The experiments of contamination with heavy metal conducted between March-October 2018, were performed for a number of 7 vegetables specified in Table 2.1 and 4 fruits specified in Table 2.2.

Table 2.1 Classification of the studied vegetable plants [9]

Group	**Common name**	**Scientific name of the plant**
Cucurbitaceae vegetables	Cucumber	*Cucumis sativus* L
Solano-fruit vegetables	Tomatoes	*Lycopersicon esculentum*
	Spinach	*Spinacia oleraceea* L
	Parsley	*Petroselinum crispum* L
Root vegetables	Carrot	*Dancus carota* L
	Parsley	*Petroselinum crispum* L
	Radishes	*Raphanus sativus* L

Table 2.2 Types of fruits studied

Group	**Common name**	**Scientific name of the plant**
Berries	Strawberries	*Fragaria ananassa*
	Cranberries	*Vaccinium myrtillus L.*
	Raspberry	*Rubus idaeus*
Fruit trees	Plums	*Prunus domestica*

Aspects during the conduct of experimental research

The stage of development in carrots

The stage of development in radishes



The stage of development in the leaves and roots of parsley



The stage of development in spinach



The stage of development in cucumbers



The stage of development in tomatoes



The stage of development in strawberries

The stage of development in blueberries

The stage of development in raspberries

The stage of development in plums

Materials and methods used

General principles of experimentation

The content of heavy metals Cu, Pb, Zn from the ash of samples prepared according to the type of material analyzed (soil, plants,) is determined using the atomic absorption spectrophotometer [14,15,16].

The methods and techniques chosen are in accordance with the recommendations elaborated by the Institute of Pedological and Agrochemical Research of Bucharest, the Decisions of the Government of Romania and the Romanian Standards regarding the elaboration of the pedological and agrochemical studies [17,18,19,20,21,22].

The methods of analysis were chosen according to the purpose pursued, namely: to assess the level of accumulation of heavy metals at the soil level - plants.

In order to track the variation of the transfer coefficient at the soil level – plants, analysis were performed for soil and vegetables, respectively fruits.

Actual development of experiments

All types of plants were planted in the soil and contaminated with the following four concentrations of heavy metal: 1.5%, 3.0%, 4.5%, 6.0% and the heavy metals used were: copper, lead, zinc, while for fruits were also mixtures of all three metals and four concentrations.

In the experiments with vegetables, the heavy metal loading was done only through the initial loading without further supplementation until harvesting.

The plants (vegetables and fruits) in the study were planted in a controlled environment using pots in which was added soil contaminated with four concentrations of Cu, Pb, Zn.

The solutions with concentrations of 1.5, 3.0, 4.5 and 6.0% were prepared individually using as reagent copper sulphate, lead acetate and Zn sulphate, and the solvent used in the preparation of the solutions was distilled water.

To obtain the of the solutions of Cu, Pb, Zn for each of the concentrations of 1.5, 3.0, 4.5 and 6.0% prepared individually, equal parts of each solution were taken, element respectively concentration and were mixed until homogenization, thus resulting the mixture.

The pots in which vegetable seedlings were planted were loaded with fertile soil that was mixed and homogenized in turn with each of the four solutions of different concentrations. For each pot the soil added was 250 ml mixing solution of Cu, Pb, Zn for 1 kg of soil.

In the , the heavy metal loading was done by the initial loading of the soil with each of the four concentrations of Cu, Pb, Zn mixing, without further supplementation until harvesting.

Compared to the soil contaminated with the four solutions of different concentrations, in parallel as reference samples, seedlings were planted in pots with uncontaminated fertile soil.

The physico-chemical properties of the uncontaminated soil (considered as the control sample) were: pH 5.0-7.0; total nitrogen 1.9%; total phosphorus 0.5%; total potassium 0.9%; electrical conductivity 1.2; particle elements over 20 mm maximum 5%, moisture 14.7%

In the pots, 3 years old plums were planted and bushes of blueberry and raspberries and strawberry stalks were 1 year old at the time of planting.

In fruits, the solution was injected in 2-3 doses, thus: each 57 ml solution in 2 doses at 21 days interval in raspberries (harvested 30 days after applying the first dose); each 57 ml solution in 2 doses at strawberries at 7 days interval (harvested 12 days after applying the first dose); each

27 ml solution in 2 doses to blueberries at 21 days intervals (less solution because they were cultivated in peat which was lighter than the universal soil used in other bushes); harvesting was done 25 days after the first dose of solution was applied); each 370 ml in plums in 3 doses at 21 days interval, harvesting 40 days after the last dose.

The determination of Cu, Pb, Zn from the contaminated soil and from the leaves and roots of the studied vegetables and fruits was performed by the spectrophotometric method (atomic absorption in the flame).

Activity 2.2. – Development of mathematical models

Processing of experimental data recorded in vegetables

Study of the variation of the final concentration in plants based on the initial concentration of heavy metal in the soil

The experimental data obtained numerically were concentrated in graphical form in representations like those given below. Similar representations exist for all the vegetables specified in Table 2.1.

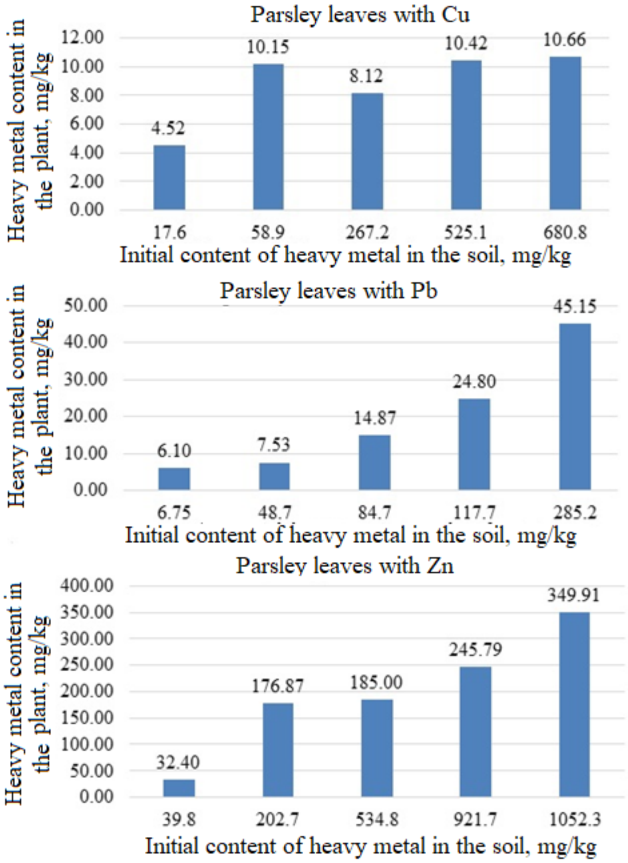

Fig. 3.1.2. Variation of the content of Cu, Pb, Zn in parsley leaves depending on the content of Cu, Pb, Zn in the soil

Study of the transfer coefficient to vegetables

The transfer coefficient studied in this chapter reflects the the heavy metal uptake ability from the soil by the plant as a function of the heavy metal concentration in the soil.

The definition formula is:

$$C_t = \frac{C_{fp}}{C_{is}}. \tag{3.1}$$

For vegetables, the variation of the transfer coefficient, C_t depending on the initial concentration of heavy metal in the soil, C_{is} is represented graphically in the following Figures:

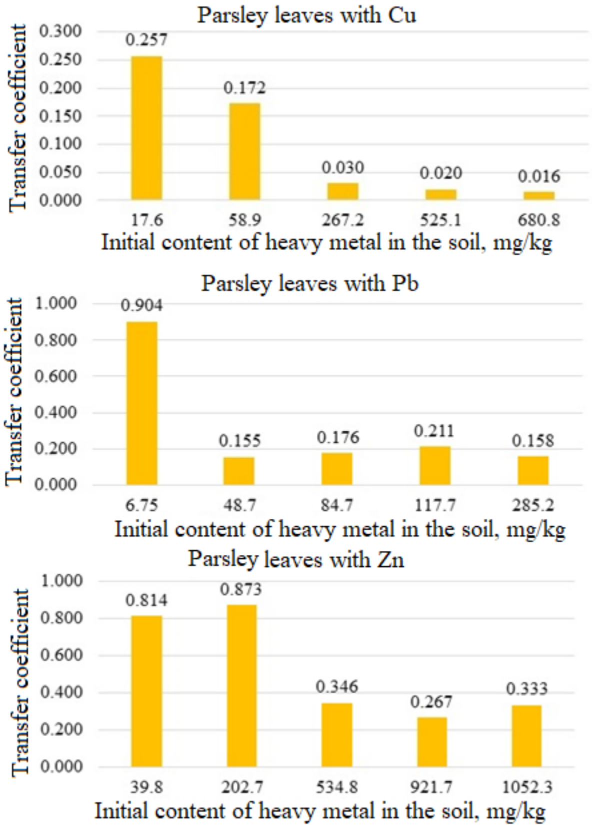

Fig. 3.2.2. Variation of the transfer coefficient for parsley leaves planted in soil contaminated with Cu, Pb, Zn

Statistical models regarding the phenomenon of accumulation of heavy metals in vegetables

Using the numerical data from the experiments and graphically represented above (as in subchapters 3.1, 3.2), the statistical mathematical models were obtained by interpolation.

- Interpolation formulas for the amount of heavy metal accumulated at the end of vegetation period

The general form of the interpolation polynomials (degrees one - four) is:

(3.2)

$$C_p(C_{is}) = c_0 + c_1 C_{is} + c_2 C_{is}^2 + c_3 C_{is}^3 + c_4 C_{is}^4$$

Table 3.3. The interpolation equations corresponding to the vegetables

Vegetable	Metal	The coefficients of the interpolation polynomials					Error*,%
		c_0	**c_1**	**c_2**	**c_3**	**c_4**	
Carrot	Cu	8.128	$3.867\cdot10^{-3}$	0	0	0	15.069
		8.119	$3.985\cdot10^{-3}$	$-1.745\cdot10^{-7}$	0	0	15.068
		8.406	$-4.221\cdot10^{-3}$	$3.138\cdot10^{-5}$	$-3.005\cdot10^{-8}$		14.225
		6.55	0.069	$4.497\cdot10^{-4}$	$1.01\cdot10^{-6}$	$-7.13\cdot10^{-10}$	$3.418\cdot10^{-13}$
	Pb	4.182	0.149	0	0	0	79.321
		-0.143	0.255	$-3.42\cdot10^{-4}$	0	0	74.097
		9.338	-0.465	$8.131\cdot10^{-3}$	$-2.129\cdot10^{-5}$	0	41.472
		-0.224	1.015	-0.029	$2.638\cdot10^{-4}$	$-6.097\cdot10^{-7}$	$2.269\cdot10^{-12}$
	Zn	33.157	0.083	0	0	0	37.801
		23.598	0.153	$-6.353\cdot10^{-5}$	0	0	33.614
		16.381	0.261	$-3.304\cdot10^{-4}$	$1.655\cdot10^{-7}$	0	31.78
		-6.277	0.759	$2.493\cdot10^{-3}$	$3.297\cdot10^{-6}$	$-1.438\cdot10^{-9}$	$1.107\cdot10^{-12}$
Parsley leaves	Cu	6.938	$5.925\cdot10^{-3}$	0	0	0	44.074
		6.603	0.011	$-6.986\cdot10^{-6}$	0	0	43.437
		6.084	0.026	$-6.411\cdot10^{-5}$	$5.441\cdot10^{-8}$	0	42.371
		0.882	0.231	$1.412\cdot10^{-3}$	$2.968\cdot10^{-6}$	$1.998\cdot10^{-9}$	$1.142\cdot10^{-12}$
	Pb	3.636	0.148	0	0	0	27.501
		2.875	0.166	$-6.016\cdot10^{-5}$	0	0	27.014
		6.903	-0.14	$3.54\cdot10^{-3}$	$-9.047\cdot10^{-6}$	0	0.7
		6.747	-0.116	$2.94\cdot10^{-3}$	$-4.396\cdot10^{-6}$	$-9.944\cdot10^{-9}$	$2.548\cdot10^{-13}$
	Zn	65.286	0.241	0	0	0	46.616
		60.405	0.277	$-3.245\cdot10^{-5}$	0	0	46.484
		-15.377	1.409	$-2.834\cdot10^{-3}$	$1.738\cdot10^{-6}$	0	8.212
		-30.093	1.733	$4.238\cdot10^{-3}$	$3.772\cdot10^{-6}$	$-9.342\cdot10^{-10}$	$2.966\cdot10^{-13}$
Cucumbers	Cu	5.647	$6.269\cdot10^{-3}$	0	0	0	19.454
		5.284	0.011	$-7.583\cdot10^{-6}$	0	0	17.047
		4.498	0.034	$-9.401\cdot10^{-5}$	$8.232\cdot10^{-8}$	0	3.285
		4.847	0.02	$3.612\cdot10^{-6}$	$-1.131\cdot10^{-7}$	$1.34\cdot10^{-10}$	$1.053\cdot10^{-13}$
	Pb	7.789	0.018	0	0	0	74.402
		4.457	0.1	$-2.635\cdot10^{-4}$	0	0	58.818
		0.164	0.426	$-4.1\cdot10^{-3}$	$9.641\cdot10^{-6}$	0	9.272
		-0.86	0.584	$8.039\cdot10^{-3}$	$4.016\cdot10^{-5}$	$-6.526\cdot10^{-8}$	$5.97\cdot10^{-13}$
	Zn	30.884	0.066	0	0	0	14.042
		33.759	0.045	$1.911\cdot10^{-5}$	0	0	12.642
		28.007	0.13	$-1.935\cdot10^{-4}$	$1.319\cdot10^{-7}$	0	7.389
		32.489	0.032	$2.342\cdot10^{-4}$	$-4.875\cdot10^{-7}$	$2.845\cdot10^{-10}$	$4.609\cdot10^{-13}$

- Graphical representations of polynomial interpolations of the heavy metal content in the plant at harvesting depending on the heavy metal content in the soil

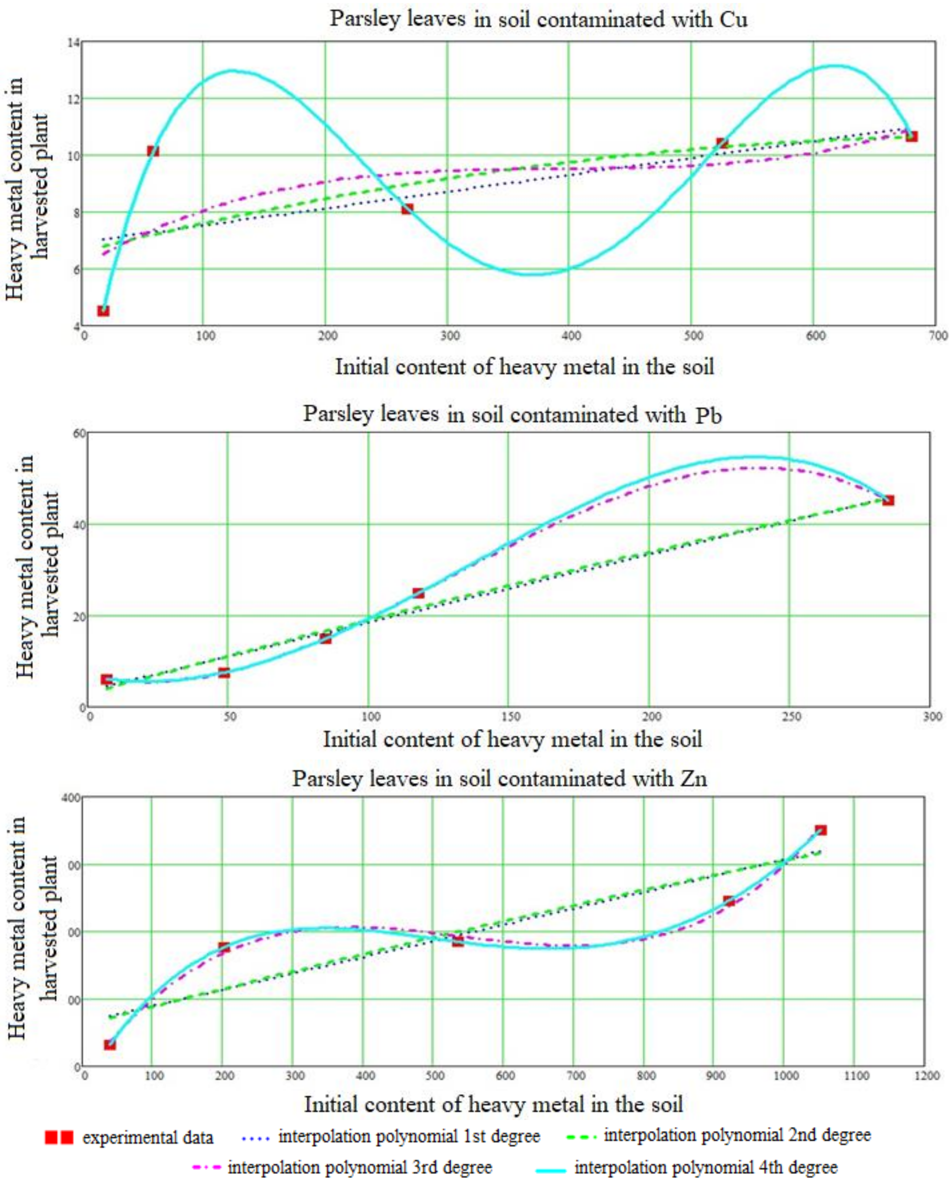

Fig. 3.3.2. Graphical representations of the interpolations of the heavy metal content in the plant (parsley leaves) at harvest depending on the heavy metal content in the soil

Similar representations exist for all the vegetables specified in Table 2.1.

Interpolation formulas for the transfer coefficient

The transfer coefficient was defined in formula (3.1). For this coefficient, outside the four interpolation curves (where there are), similar to formula (3.2), which refers to the heavy metal content accumulated by the plant at the end of the vegetation period:

$$C_t(C_{is}) = c_0 + c_1 C_{is} + c_2 C_{is}^2 + c_3 C_{is}^3 + c_4 C_{is}^4 \tag{3.3}$$

The coefficients c_0 to c_4 will be tabulated similar to those given for C_p interpolation.

For the transfer coefficient, the way in which the experimental data is placed, shows a possible monotonous, possibly asymptotic, mathematically modelable descent according to the formula:

$$C_t(C_{is}) = \frac{c_0}{C_{is}} + c_1 \tag{3.4}$$

The coefficients of the polynomials (3.3), shown in Table 3.4, and those of the hyperbola (3.4), shown in Table 3.5, were entered in the Tables similar to the Table in subchapter 3.4.1.

Table 3.4. The coefficients of the interpolation polynomials

Vegetable	Metal	Coefficients of the interpolation polynomials					Error*,%
		c_0	c_1	c_2	c_3	c_4	
Carrot	Cu	0.277	$-4.713\cdot10^{-4}$	0	0	0	175.587
		0.362	$-1.67\cdot10^{-3}$	$1.762\cdot10^{-6}$	0	0	122.469
		0.462	$-3.508\cdot10^{-3}$	$8.829\cdot10^{-6}$	$-6.731\cdot10^{-9}$	0	93.579
		0.598	-0.01	$5.342\cdot10^{-5}$	$-1.031\cdot10^{-7}$	$6.608\cdot10^{-11}$	$5.909\cdot10^{-13}$
	Pb	0.458	$-1.402\cdot10^{-3}$	0	0	0	158.923
		0.712	$-7.62\cdot10^{-3}$	$2.01\cdot10^{-5}$	0	0	113.81
		0.976	-0.028	$2.556\cdot10^{-4}$	$-5.917\cdot10^{-7}$	0	1.025
		0.972	-0.027	$2.419\cdot10^{-4}$	$-4.861\cdot10^{-7}$	$-2.259\cdot10^{-10}$	$3.623\cdot10^{-13}$
	Zn	0.446	$-3.642\cdot10^{-4}$	0	0	0	60.035
		0.545	$-1.094\cdot10^{-3}$	$6.61\cdot10^{-7}$	0	0	16.433
		0.571	$-1.472\cdot10^{-3}$	$1.596\cdot10^{-6}$	$-5.799\cdot10^{-10}$	0	10.878
		0.546	$-9.403\cdot10^{-4}$	$-7.101\cdot10^{-7}$	$2.759\cdot10^{-9}$	$-1.534\cdot10^{-12}$	$1.934\cdot10^{-13}$
Parsley leaves	Cu	0.2	$-3.272\cdot10^{-4}$	0	0	0	112.486
		0.252	$-1.06\cdot10^{-3}$	$1.077\cdot10^{-6}$	0	0	47.272
		0.279	$-1.833\cdot10^{-3}$	$4.051\cdot10^{-6}$	$-2.832\cdot10^{-9}$	0	16.952
		0.303	$-2.759\cdot10^{-3}$	$1.014\cdot10^{-5}$	$-1.598\cdot10^{-8}$	$9.02\cdot10^{-12}$	$4.973\cdot10^{-13}$
	Pb	0.5	$-1.654\cdot10^{-3}$	0	0	0	171.259
		0.824	$-9.559\cdot10^{-3}$	$2.555\cdot10^{-5}$	0	0	106.208
		1.07	-0.028	$2.461\cdot10^{-4}$	$-5.541\cdot10^{-7}$	0	31.212
		1.184	-0.046	$6.82\cdot10^{-4}$	$-3.932\cdot10^{-6}$	$7.223\cdot10^{-9}$	$2.63\cdot10^{-12}$
	Zn	0.853	$-5.935\cdot10^{-4}$	0	0	0	49.634
		0.964	$-1.404\cdot10^{-3}$	$7.339\cdot10^{-7}$	0	0	39.635
		0.824	$6.817\cdot10^{-4}$	$-4.426\cdot10^{-6}$	$3.201\cdot10^{-9}$	0	23.812
		0.71	$3.178\cdot10^{-3}$	$-1.526\cdot10^{-5}$	$1.889\cdot10^{-8}$	$-7.205\cdot10^{-12}$	$7.119\cdot10^{-13}$
Cucumbers	Cu	0.19	$-3.136\cdot10^{-4}$	0	0	0	169.64
		0.245	$-1.094\cdot10^{-3}$	$1.148\cdot10^{-6}$	0	0	123.307
		0.286	$-2.288\cdot10^{-3}$	$5.738\cdot10^{-6}$	$-4.372\cdot10^{-19}$	0	99.395
		0.415	$-7.355\cdot10^{-3}$	$3.904\cdot10^{-5}$	$-7.634\cdot10^{-8}$	$4.936\cdot10^{-11}$	$4.653\cdot10^{-12}$
	Pb	0.31	$-1.142\cdot10^{-3}$	0	0	0	89.319
		0.435	$-4.183\cdot10^{-3}$	$9.829\cdot10^{-6}$	0	0	6.995
		0.433	$-4.033\cdot10^{-3}$	$8.062\cdot10^{-6}$	$4.44\cdot10^{-9}$	0	6.853
		0.418	$-1.798\cdot10^{-3}$	$-4.75\cdot10^{-5}$	$4.349\cdot10^{-7}$	$-9.206\cdot10^{-10}$	$7.649\cdot10^{-13}$
	Zn	0.587	$-5.594\cdot10^{-4}$	0	0	0	155.184
		0.831	$-2.355\cdot10^{-3}$	$1.626\cdot10^{-6}$	0	0	91.914
		1.013	$-5.077\cdot10^{-3}$	$8.362\cdot10^{-6}$	$-4.179\cdot10^{-9}$	0	48.423
		1.136	$-7.765\cdot10^{-3}$	$2.002\cdot10^{-5}$	$-2.107\cdot10^{-8}$	$7.757\cdot10^{-12}$	$1.062\cdot10^{-12}$

Table 3.5. The hyperbolic interpolation equations corresponding to the transfer coefficient

Vegetable	Metal	Hyperbola coefficients	
		c_0	**c_1**
Carrot	Cu	7.551	$9.208 \cdot 10^{-3}$
	Pb	4.446	0.134
	Zn	15.419	0.141
Parsley leaves	Cu	4.349	0.029
	Pb	5.255	0.119
	Zn	18.877	0.398
Cucumbers	Cu	5.075	0.01
	Pb	2.102	0.105
	Zn	31.551	0.064

- Graphical representations of the polynomial interpolations for the transfer coefficient

The graphs were made similar to those in subchapter 3.4.2 with the heavy metal content in the plant at the end of the vegetation period, further containing the hyperbolic interpolation curve.

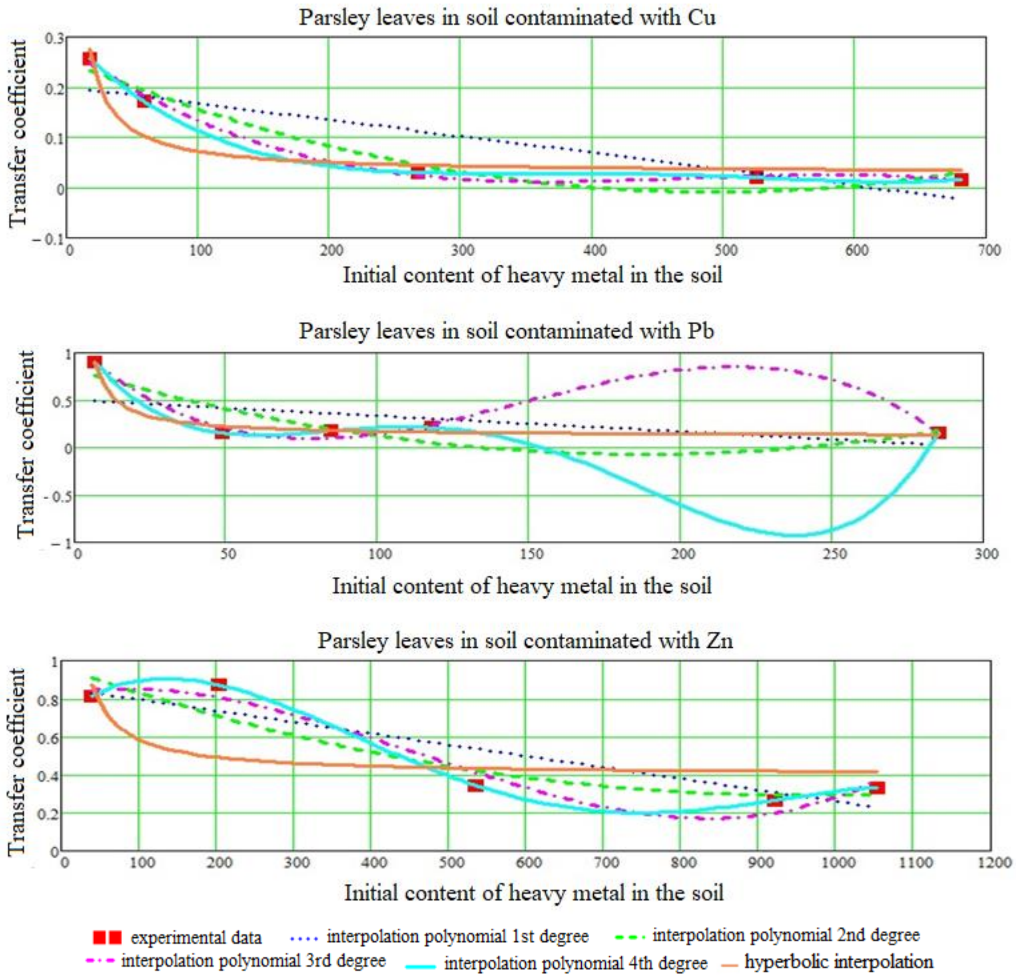

Fig. 3.3.5. Graphical representations of the interpolations for the transfer coefficient to parsley leaves

Similar representations exist for all the vegetables specified in Table 2.1.

- Study of the variation of the final concentration in fruit depending on the initial concentration of heavy metal injected into the soil

Figure 4.1 shows the variations in the content of heavy metals: Cu, Pb, Zn in strawberry and raspberries fruits grown in soil injected with heavy metal solutions separated by different concentrations.

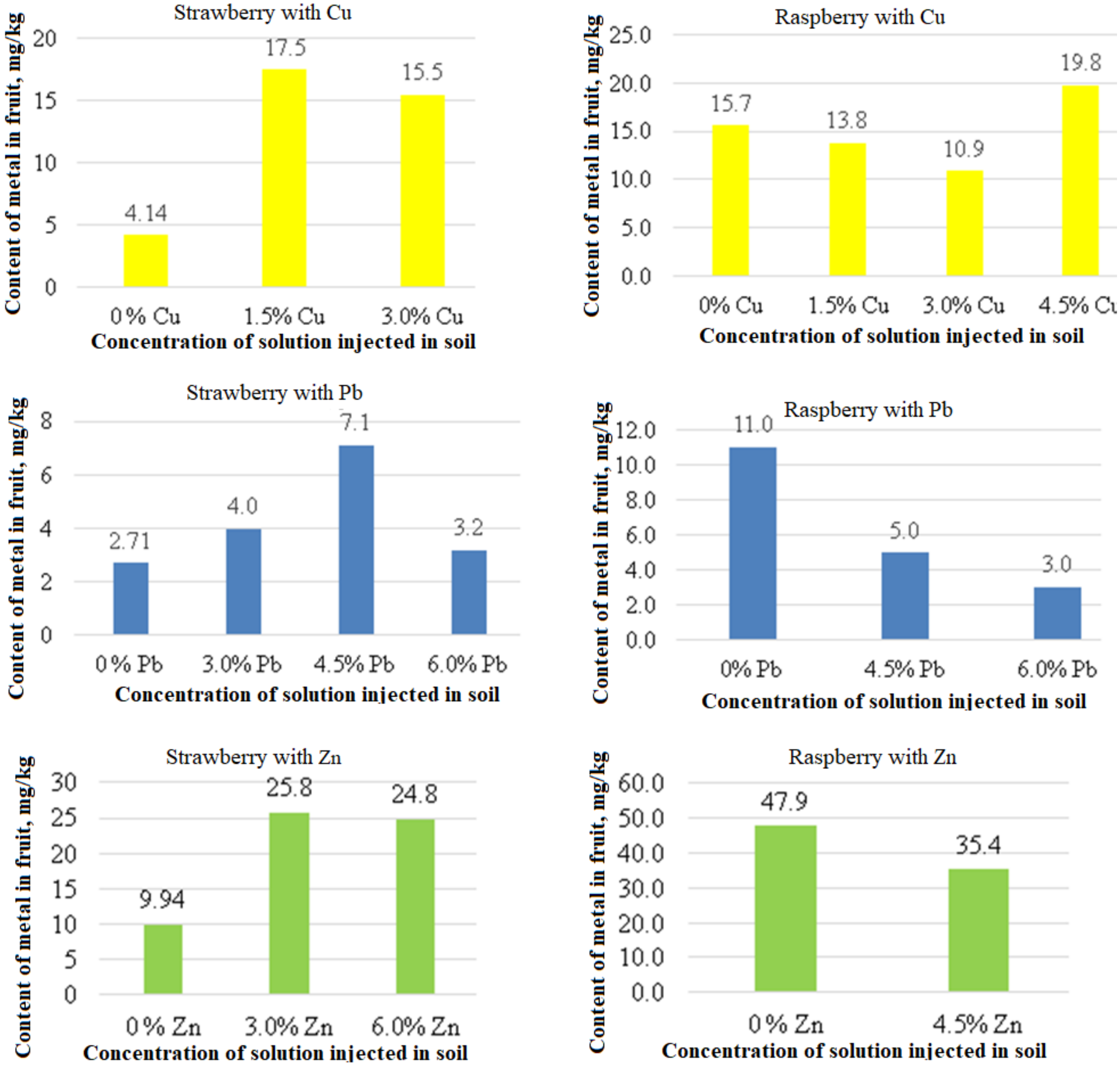

Fig. 4.1. Variation of Cu, Pb and Zn content in strawberry and raspberry fruits depending on the metal content in the soil

The general form of the interpolation polynomial (grades one - two) is:

$$C_f(C_{is}) = c_0 + c_1 C_{is} + c_2 C_{is}^2 \tag{3.5}$$

Table 3.6. The interpolation equations corresponding to the fruits

Fruit	Metal	Coefficients of the interpolation polynomials			
		c_0	c_1	c_2	c_3
Strawberries	Cu	4.14	14.027	-3.413	-
	Pb	2.71	-4.979	2.763	-0.32
	Zn	9.94	18.379	-6.055	0.567
Raspberry	Cu	15.7	1.911	-3.067	0.632
	Pb	4.14	14.027	-3.413	-
	Zn	47.9	-21.289	4.114	-

- Interpolation of experimental data on fruits

The interpolation curves for strawberries are shown in Figure 4.2.

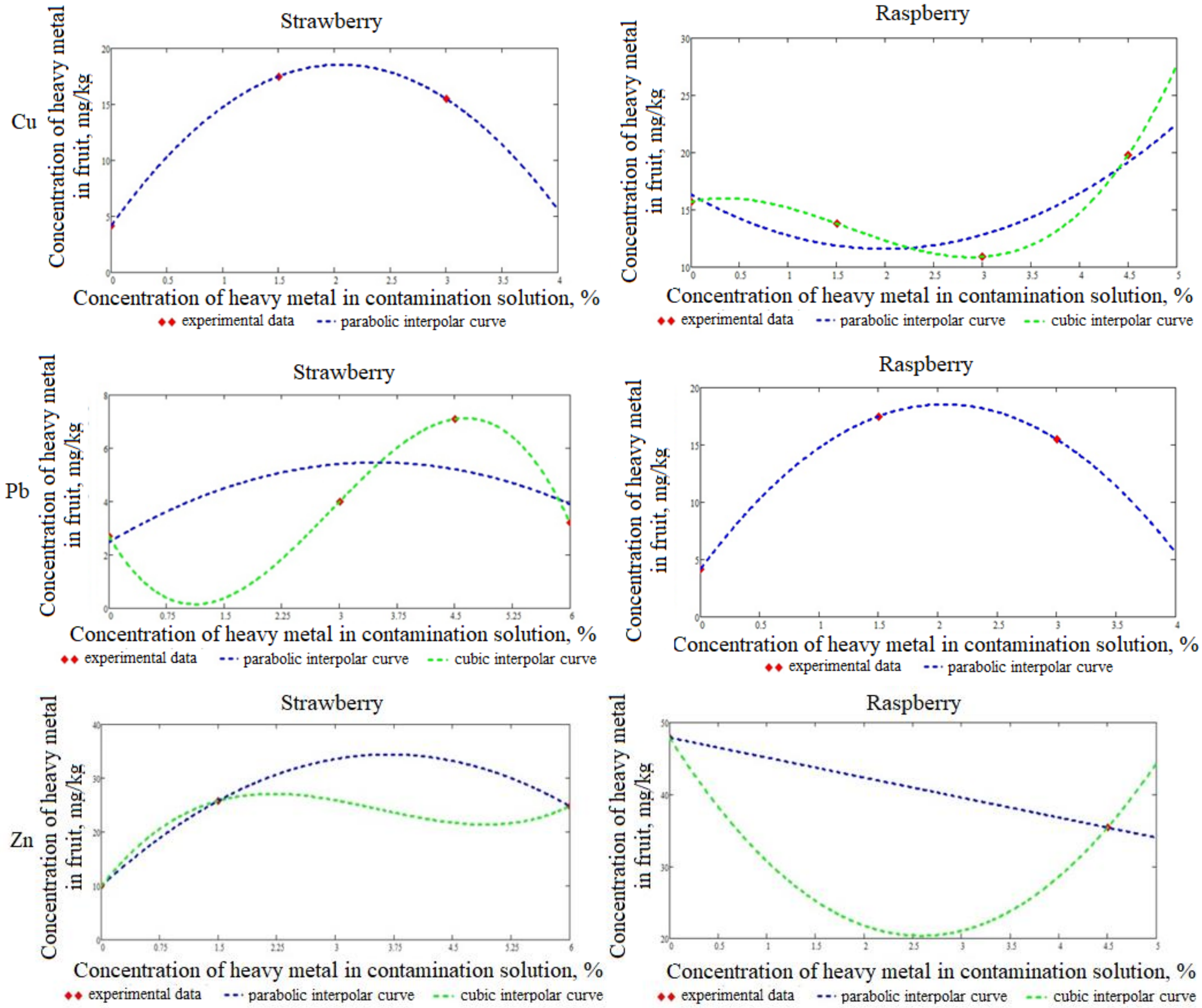

Fig. 4.2. Graphical representations of the interpolations for the contents of Cu, Pb and Zn in strawberry and raspberry fruits

Similar representations, but for soil contaminated with heavy metals (Cu, Pb, Zn), exist for all the fruits specified in Table 2.2.

CONCLUSIONS

Conclusions on the behavior of vegetables

- Considering all the cases of vegetables grown with different concentrations of heavy metal in the soil it is observed that 57% have a tendency to increase the amount of heavy metal accumulated in the plant, with the increase of the initial concentration of heavy metal in the soil.

- On plants, the situation is described in Table 5.1. In general, the conclusion regarding the increasing monotony of the curves representing the variation of the heavy metal concentration in the plant remains valid. Also from Table 5.1 it is observed that the curves of variation of the concentration of heavy metal in plants that are not monotonous, but they appear more often in the case of contaminations with copper, and less with lead and zinc.

Table 5.1. Variation of the heavy metal concentration dependence curves in the plant and soil by categories of vegetables

Vegetable	Percentage of rising curves with the initial concentration of heavy metal in the soil	Percentage of oscillating curves with the initial concentration of heavy metal in the soil
Carrot	**66.67** (Pb, Zn)	**33.33** (Cu)
Radishes	**100** (Cu, Pb, Zn)	-
Parsley root	**66.7** (Pb, Zn)	**33.33** (Cu)
Parsley leaves	**66.7** (Pb, Zn)	**33.33** (Cu)
Spinach	**66.7** (Pb, Zn)	**33.33** (Cu)
Cucumbers	**66.7** (Cu, Zn)	**33.33** (Pb)
Tomatoes	-	**100** (Cu, Pb, Zn)

- Considering all the cases of vegetables grown with different concentrations of heavy metal in the soil, it is observed that 76% have a decreasing tendency of the coefficient of transfer of the heavy metal from the soil to the plant, with the increase of the initial concentration of heavy metal in the soil. As a general conclusion it would result that for the studied plants the heavy metal bioaccumulation is the lower the higher the initial concentration of heavy metal in the soil. This means that as the soil will be more heavily contaminated with heavy metals, the fitoremediation of the soil with plants of the studied or similar category will be more difficult.

In the other cases there are some minimums and maximums, which can represent accidents that can be avoided only by performing a large number of repetitions for the same experimental case.

- On vegetables, the situation is described in Table 5.2. In general, it remains valid the conclusion regarding the increasing monotony of the curves representing the variation of the heavy metal concentration in the vegetable. Also, from Table 5.2 it is observed that the curves of variation of the concentration of heavy metal in vegetables that is not monotonous and appear more often at contaminations with copper, and less at contaminations with lead and zinc.

Table 5.2. Variation of the curves of dependence of the heavy metal transfer coefficient from soil to vegetables

Vegetable	Percentage of decreasing curves with the transfer coefficient of the heavy metal from the soil to the plant	Percentage of oscillating curves with the transfer coefficient of heavy metal from the soil to the plant
Carrot	**100** (Cu,Pb, Zn)	**0**
Radishes	**66.7** (Cu, Pb, Zn)	**33.33** (Zn)
Parsley root	**66.7** (Cu, Zn)	**33.33** (Pb)

Parsley leaves	**33.33** (Cu)	**66.7** (Pb, Zn)
Spinach	**100** (Cu,Pb, Zn)	**0**
Cucumbers	**100** (Cu,Pb,Zn)	**0**
Tomatoes	**66.7** (Pb,Zn)	**33.33** (Cu)

A conclusion that is easily observed is that the vegetation period (harvesting, the period established by the conventional agronomic operator) is constant for: spinach, radishes, carrots, parsley, and that it varies slightly for tomatoes and cucumbers. These slight variations could be related to the absorption of heavy metal, but for this conclusion to gain certainty additional experiences must be made.

Conclusions on the behavior of fruit

The experiences of accumulation of heavy metals in fruits are more difficult because in general, the distribution of the heavy metal in the plant should be studied on parts (root, stem, branches with and without fruits, leaves, fruits, seeds, etc.). This mode of analysis requires a large number of complicated analyzes for a single plant. Analyzing the concentration of heavy metals in the plant only in the fruit at the complete harvesting stage, we could only draw some conclusions of intermediate character.

- For blueberries it was observed that the transfer rate decreases with increasing heavy metal concentration in the soil (mixture); of the most absorbed metals is Zn, then Cu and finally Pb.

- Also, in case of blueberries, the most intense absorption is observed for the lowest concentration in heavy metal (contamination concentration 0, does not mean that the soil did not contain heavy metals, the soil used had an initial heavy metal concentration), similar case for the high raspberry in soil contaminated separately with Pb, respectively with Zn, and contrary to the case of contamination of copper soil. It has been observed that in the initial mixture there is a ratio of ¼ Cu, ½ Pb and ¼ Zn, and the transfer coefficients show that blueberries absorb much more zinc than lead and copper. Moreover, lead is the hardest absorbed.

- For blueberries it was observed that the initial mixture of heavy metals in the soil was, in general, the same for all solutions prepared for contamination (1.5, 3.0, 4.5, 6.0%). Knowing the amount of metal in the absorbed mixture, one can calculate the correlation between the structure of the contamination mixture and the structure of the absorbed mixture of metals. All correlations are negative and relatively significant. This means that blueberries extract zinc as a priority (this was not the metal with the highest concentration in the contamination mixture). Therefore, it is likely that the plant will have its own filter mechanism for certain heavy metals and will extract those metals with priority. To consolidate this conclusion, we recommend several diversified experiments.

- It was observed that the variation of the heavy metal content in strawberries (fruit) does not recommend linear interpolation (linear regression), as these distributions have potential extreme points (minimum or maximum).

- The final concentrations in the plant (strawberry fruits) and the initial concentrations in the soil, of the heavy metals were well correlated for the contamination concentration 0% and 6.0%, the medium correlated for the concentration of 3.0% and weakly correlated for the concentration of 1.5% and 4.5%. The meaning of a good correlation between the two categories of concentrations is that the order of the concentrations of the metals in the soil mixture is kept at the concentrations for each metal in the plant.

- For the experiments made on strawberries with only one heavy metal (Cu, Pb, Zn), although only three concentrations of heavy metal were examined in the soil it was observed that in each of the three cases the variation of the final content in the plant has a maximum. The maximum is located within the experimental range. This would mean that as the concentration increases, the heavy metal absorption capacity in the soil decreases (for strawberries), which could lead to explanations related to the plant's possibility to develop protective mechanisms.
- In the case of plums (fruits of the plum) a more pronounced absorption of Zn, then of Cu and less of Pb was observed, both for the case of soil contamination with a single type of metal, as well as in the case of soil infestation with the mixture of the three metals (Cu, Pb, Zn).
- Behavior of fruits in soils contaminated with a mixture of heavy metals to a certain extent to ascertain to what extent the fruits behave as heavy metal filters by preferentially selecting some of them.

Observation. *Following the study of growing fruit in soil contaminated with heavy metals, it is recommended to conduct complex experiments for several seasons in order to determine the behavior of plants for several generations in order to recover some plants affected by heavy metals, or which have functioned for a long time with phytoremediation role.*

Conclusions regarding the statistical mathematical models

These conclusions refer to the statistical mathematical models elaborated by the interpolation of the experimental data.

- Polynomial representations are a primary and common form of statistical models with practical applications in both scientific research and agricultural management. Using such formulas it is possible to calculate the necessary plants for phytoremediation or to classify the production within the limits stipulated by the health standards. Using the same formulas, one can optimize phytoremediation processes or optimal identification of crop plots, considering the use for marketing / consumption or phytoremediation processes.
- Interpolation polynomials of degrees one and two are the most usable because they have small variations between experimental points. The third and fourth degree polynomial curves show large relative variations between the experimental points and following their use presents the risk of producing large errors, although for example, restricting us only to the set of experimental points, the fourth degree polynomial passes exactly through the experimental points.
- In general, the interpolation curves show the tendency of increasing the final concentration of heavy metal in the plant (at the final stage of vegetation / harvesting) and decreasing the coefficient of heavy metal transfer in the plant at the end of the experiments.
- For the transfer coefficient the experimental data presented in subchapter 3.2, showed a pronounced tendency to hyperbolic decrease of this coefficient with the initial concentration of heavy metal in the soil. Hence, a hyperbolic interpolation formula (3.4) was considered, which proves to be adequate with some exceptions where the experimental data do not notice the above mentioned tendency.

-The statistical mathematical models provided by formulas (3.2), (3.3) and (3.4) whose generic coefficients, for each particular case, are given in Tables 3.3, 3.4 and 3.5, can be used for interpolation (calculation of any heavy metal concentration in the plant or transfer coefficient, only in the experimental range considered for the initial concentration of heavy metal in the soil). Extrapolation of this data is not recommended, the use of formulas for this purpose being made with the exclusive responsibility of the users.

-. Regarding the interpolation models for fruits, these are less documented because it was worked with a number of four concentrations (on certain categories of concentrations the plants did not grow). For strawberries as for raspberries, in these circumstances we recommend maximum degree 2 interpolation curves, because the experimental distributions have maximum or minimum (they are not linear). There have also been tried interpolation of degree 3 (polynomial curves of degree 3), which even go through the experimental points, so they have a total null error with the experimental data, it shows aberrant behavior between the experimental points.

General conclusions

Following the experiments and the statistical processing of their results, as general phenomenology is noted:

- until the end of the vegetation (harvesting) stage the plants considered in experiments continuously accumulate (monotonous growth) heavy metals from the contaminated soil;
- the transfer coefficient decreases with the increase of the heavy metal concentration in the soil, at least until the end of the vegetation period (harvesting).

As a result of the obtained results, it is recommended to resume the experiments developed in the second phase for a maximum number of 2 plants (vegetables) with harvesting in time from sowing until the death of the plant (with the retention of the seeds produced by the plant). This type of experiment must be done with repetitions to achieve an acceptable degree of trust by the specialized magazines for publication. The experiments achieved in this phase are only demonstrative, because the degree of trust cannot be estimated.

The results obtained for vegetables show that such experiences and statistical modeling can provide the basis for the construction of dynamic mathematical models that can simulate the life of a plant, even of several generations of plants, and highlight possible self-defense mechanisms developed by plants, adaptations to new conditions / environmental variables (climate change), possibilities of time recovery of the qualities of some plants.

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PHASE 3 - DEVELOPMENT OF A METHOD FOR IMPROVING PARAMETERS TO AMELIORATE SOIL CONTAMINATION

Activity 3.1. – Experiments in the laboratory / field

1. Experimental research on the accumulation of heavy metals over time in green salad

The experiments conducted consisted in the development and monitoring of a green salad culture for a period of time equal to 139% of the ripening or vegetation period of the crop. The culture was done in separate pots, on three categories of soil: contaminated with heavy metal (zinc), 1.5%, 3.0% and 4.5% respectively. For each of the three soil categories, 11 harvestings were carried out at approximately equal time periods (around seven days). The entire culture was developed in the greenhouse, so the temperature and moisture conditions were common for all plants and had slight variations.

Salad seedling, Fig. 1, was planted in soil contaminated with the following three zinc concentrations: 1.5%, 3.0%, 4.5%. The salads were planted in controlled environment using pots in which the contaminated soil was added to each of the three solutions with concentrations of 1.5%, 3.0%, 4.5% zinc, prepared individually and using as reagent Zn sulphate and distilled water. The pots in which the seedlings were planted were loaded with fertile soil (1 kg/pot) which was mixed and homogenized in turn with each of the three solutions of different concentrations (250 ml solution). The heavy metal loading was carried out by initially loading the soil with each of the three Zn concentrations, without further supplementation until harvesting. In parallel as reference samples, seedlings were also planted in pots with uncontaminated fertile soil.

The physico-chemical properties of the uncontaminated soil (considered as the control sample) were: pH 5.0-7.0; total nitrogen 1.9%; total phosphorus 0.5%; total potassium 0.9%; electrical conductivity 1.2; particle elements over 20 mm maximum 5%, moisture 14.7%.

Sampling of the vegetal samples was done in time up to 68 days after planting (Fig. 2) and each time the salad was harvested, the soil sample was taken from the pot, after it was homogenized.

<u>The measurement of the height and</u> diameter of each salad was done with the ruler, the height was measured from the tip of the root to the end of the last leaf, and the diameter consisted of the left-right spread of all the salad leaves on both sides of the stem and the size of the stem at the widest leaf on the right side of the stem to the widest leaf on the left.

<u>The mass of samples</u> was determined by weighing at the KERN electronic scale of precision 0.001 g.

<u>The moisture of soil and plant</u> was made using the oven where it is dried at 105 °C soil/plant to evaporate the water related to the soil/plant.

<u>Soil pH</u> was determined using a pH determination kit. Soil sample was taken about 20 g, dried in the oven and then passed through a 1 mm sieve and placed in a bowl with 100 ml of water. Stirred several times for 30 minutes, then filtered. The pH paper was used, which was immersed in the filtered liquid, then waited for 30-60 seconds and compared the resulting color with the color sample on the lid.

<u>The determination of zinc</u> from the contaminated soil and from the whole salad plant (root and leaves) was performed by the spectrophotometric method (atomic absorption in the flame) [1,2,3].

Fig. 1 - Planting the salad seedling

Harvesting 1

Harvesting 3

Harvesting 5

Harvesting 7

Harvesting 9

Harvesting 10

Fig. 2 - Pots with plants from the three types of crops harvested at several stages of harvesting

Variation of some environmental conditions during the time period of the life of culture

The variation in time of the pH and moisture of the plant and also in the soil, are given in Fig. 3, 4, 5. The relatively small interval in which these conditions were controlled can be observed.

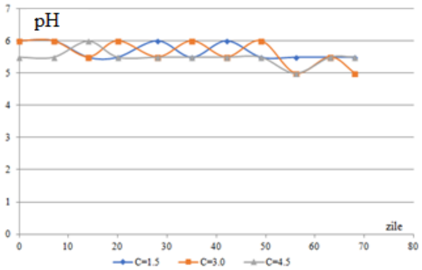

Fig. 3 - Variation in time of pH

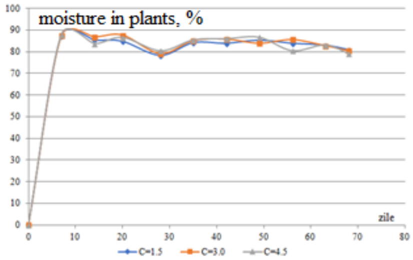

Fig. 4- Variation in time of plant moisture

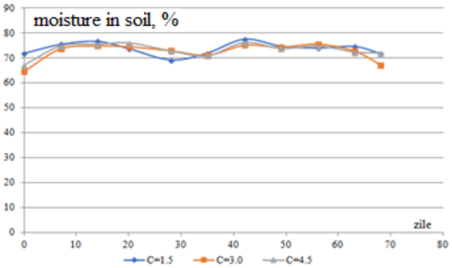

Fig. 5 - Variation in time of soil moisture

The graphical representation of the raw data recorded at each material harvest is given by categories of measured parameters, for the three crops in soil contaminated with zinc in the three concentrations. The variation of the mass of the plant harvested over time appears in Fig. 7. In Fig. 8, the variations of the heights of the collected samples, with time are shown. The variation of the diameter of the samples collected over time, in graphical form is shown in Fig. 9.

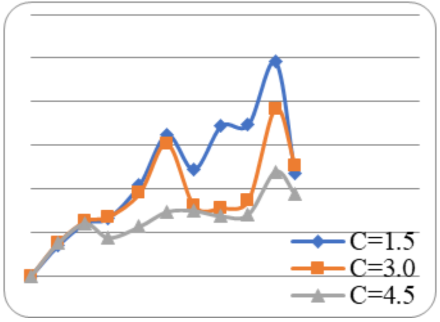

Fig. 7 - Variation in time of the mass of harvested plant

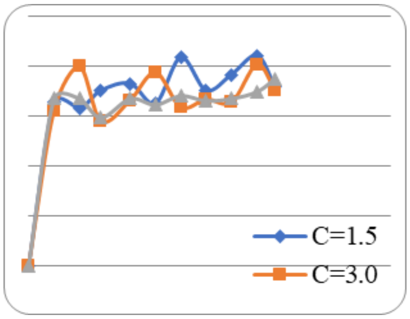

Fig. 8 - Variation in time of the height of harvested plant

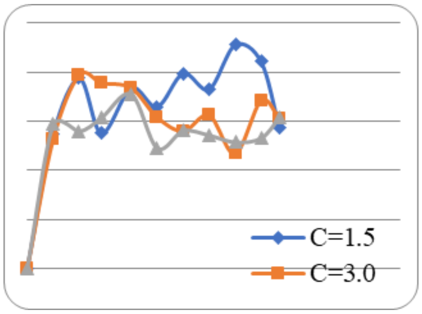

Fig. 9 - Variation in time of the diameter of harvested plant

Some statistical observations that is required:

- the variation in time of plant mass, for the three groups of plants (after the initial concentration of Zn in soil), are very well correlated with each other. Thus, the variation of masses over time is similar (the monotony intervals coincide or they are very close). The correlation between the variation of mass of the samples collected from the soil infused with Zn in the concentration 1.5% and the variation of mass of the samples collected from the soil infested with Zn in the concentration 3.5%, has the value 0.879, the correlation between the variation of the mass of the samples collected from the soil infected with Zn in the concentration 3.0% and the variation of the mass of the samples collected from the soil infused with Zn in the concentration 4.5%, has the value 0.907, and the correlation between the variation of the mass of the samples collected from the soil contaminated with Zn in the concentration 4.5% and the variation of the mass of the samples collected from the soil contaminated with Zn in the concentration 1.5%, has value 0.919.

- the order between the three curves is visible, an order that is clearly observed after approximately 14 days (28.57% of the vegetation period). The order relationship is:

$$m_{4.5}(t) \leq m_{3.0}(t) \leq m_{1.5}(t) \tag{1}$$

where $m_{1.5}(t), m_{3.0}(t), m_{4.5}(t)$ are the mass functions of the green salad grown in the soil with initial concentration 1.5, 3.0, respectively 4.5 % Zn, time-dependent functions. This means that the increase of the heavy metal content in the soil leads to the decrease of the biological mass of the plant.

- an observation that is required to be reinforced by additional experiments and by statistical mathematical modeling, is that the masses of individuals that grow in the three types of soils, increase until near the optimal age of vegetation (45-50 days) [4], approximately monotonous, after which they still grow to about 130.6% of the standard vegetation period, then, without exception, they decrease.
- until the repetitions of experiments, in which to vary the influence factors of the evolution of the plants: pH, luminosity, temperature, moisture, nutrients, etc., the minima and the maxima that disturb the monotony of the increase of the mass of the plants, can be attributed to the influence factors underlined.

Other observations will be completed in the data processing section, respectively statistical mathematical modeling.

- as for the geometrical characteristics of the plants, the behavior of both (the height and the generalized diameter), varies quickly to an average height (in about 25% of the vegetation period), after which it varies in a narrow range around this value.
- the order of the three types of green salad culture, for the parameters of height and generalized diameter, is less clear, this can be noticed only by mediation or considerations of the intervals where there is a certain order between these parameters.
- the influence of growth parameters (medium) on the evolution of mass (recorded parameters: pH, plant moisture and soil moisture), at least in their (relatively small) variation intervals, is not obvious as it results from the correlation calculation. Specifically, pH and moisture were maintained with as little variation around an average value. (see Fig. 10).

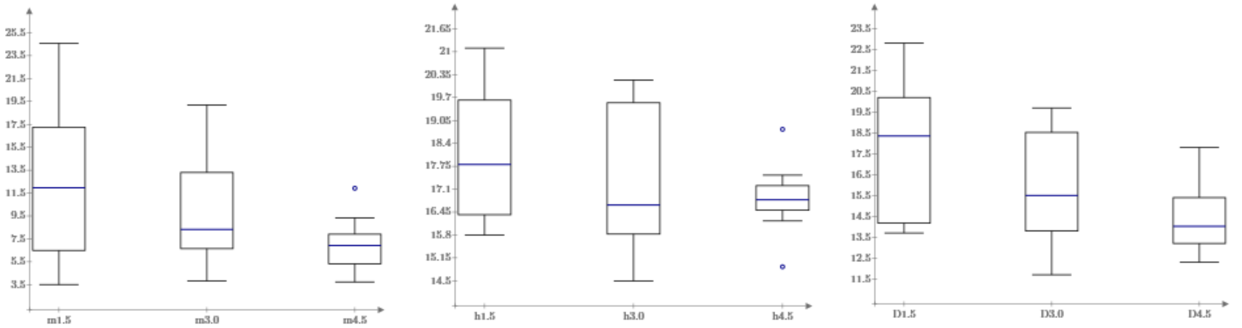

Fig. 10 - Box-plot diagrams for mass distributions, heights and diameters, for the last 10 harvests

The variation of the heavy metal content in plants is presented in graphical form in Fig. 11. Some general observations on the variation in time of the heavy metal concentration in the salad cultivated in the three categories of soils are obvious:

- globally, without altering the concentration of heavy metal in soil, the accumulation of heavy metal in plants increases;
- at local level (for certain subintervals of time) the concentration of heavy metal in plants may decrease (obviously making abstraction of some measurement or cultivation errors) - this phenomenon is interesting because, if it is real, it may indicate the fact that the removal of heavy metal from the plant can be done naturally, and future experiences are needed to determine the influential factors in this process;

- accumulation of heavy metal in plants grows from plants grown in the soil least contaminated with heavy metal to plants grown in soil with the highest initial heavy metal concentration. In 7 of the 11 harvests (63.64%) the highest concentration of heavy metal was recorded in the sample grown in soil contaminated with 4.5% solution, followed by the sample grown in soil contaminated with solution of 3.0% concentration and then by the sample grown in the soil contaminated with 1.5% concentration solution. This behavior can also be seen on the graphs in Fig. 11. Furthermore, this observation is reinforced by the average of the last 10 harvests, which has the value 228.993 mg/kg, for the culture with soil contaminated with 1.5% concentration solution, 315.334 mg/kg, for the culture with soil contaminated with concentration solution 3.0%, 357.909 mg/kg, for soil culture contaminated with 4.5% concentration solution. Therefore the same order: for soil infested with a higher concentration of heavy metal, the accumulation is more intense, at least for the range of values covered by these experiences.

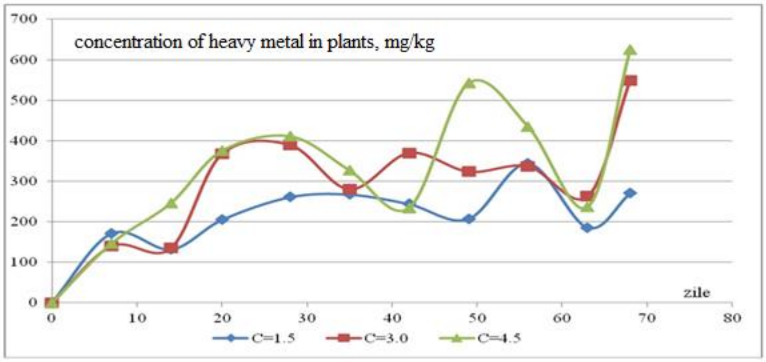

Fig. 11 - Variation in time of the concentration of heavy metal (Zn) in plants for the three cases of contamination.

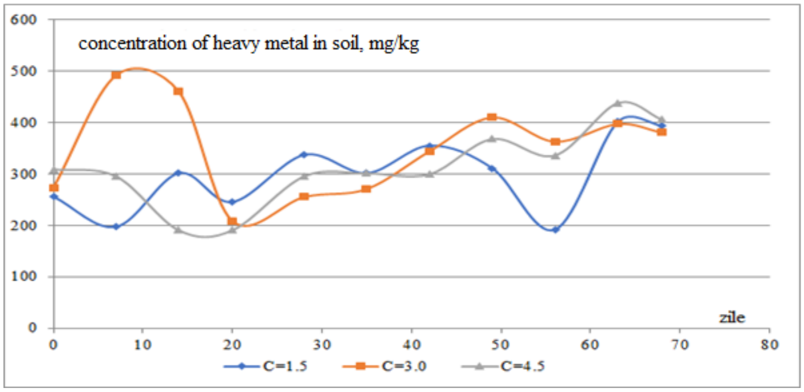

Fig. 12 - Variation in time of the concentration of heavy metal (Zn) in soil for the three cases of contamination.

It can also be seen that, according to the experimental data (the temporal variations represented in Fig. 12), the variation of the heavy metal concentration in the soil oscillates, having a slight tendency to increase over time. The question arises whether the phenomenon is real or due to measurement or interpretation errors. The ordinary reader might be tempted to accept a certain balance of heavy metal in the soil. If the concentration of heavy metal in plant is naturally increasing, local or continuous decrease may be accepted at the end of the study time period, placing this decrease phenomenon on account of a decrease of the absorption rate of metal and the increase of mass. For metal concentration in the soil, this explanation is more difficult to accept, even if some of the substances in the soil pass into the plant (the mass is considered very small compared to that of the soil).

2. Experimental research on the accumulation of heavy metals in strawberries

In the experiments on fruits conducted last year on fruit trees and bushes, in this year (2019), the experiments were continued only on bushes, respectively strawberries, because they fruit in a short interval, so that results could be obtained in the project development period.

Strawberry seedlings were planted in soils contaminated with Cu, Zn and Pb, in solutions of four concentrations each: 1.5%, 3.0%, 4.5% and 6.0%, plus 0% non-contaminated control sample.

Figure 13 shows the metal contents depending on the concentration of each metal (Cu, Zn, Pb) in the soils prepared for planting strawberry seedlings.

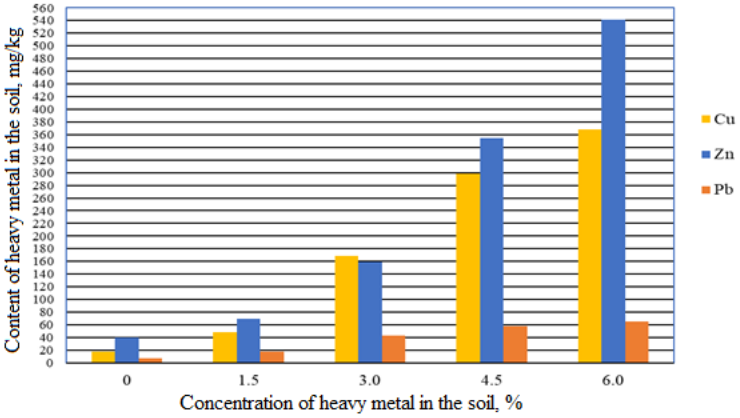

Fig. 13 – The content of Cu, Zn, Pb in soils that have developed strawberries

Figures 14, 15 and 16 show the content of heavy metals in the soil, the masses and moistures of the strawberries harvested depending on each metal, respectively the concentration of the metal.

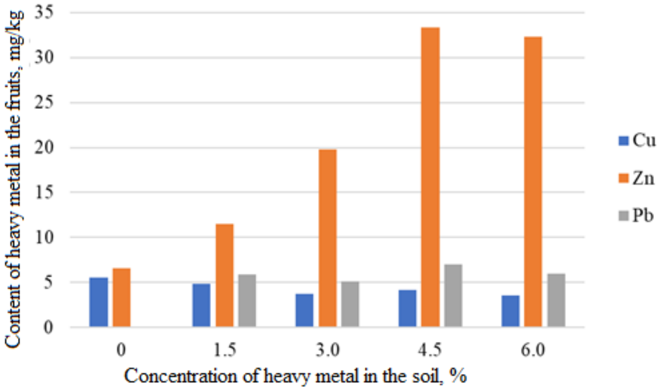

Fig. 14 – The content of metals (Cu, Zn, Pb) in strawberries (fruits) grown in soils contaminated with different concentrations of metals

From Fig. 14 it can be noticed that compared to the control sample, in which the metals are found very little (lead is even non-existent), the values increase especially in the case of zinc more and less in the case of lead. Copper is just below the limit of the control sample, this would mean that with the high concentration the heavy metal absorption capacity in the soil decreases (for strawberries), which could lead to explanations related to the possibility of the plant to develop its protective mechanisms.

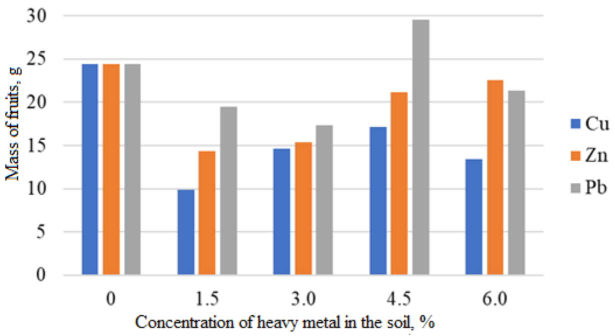

Fig. 15 – Masses of strawberries grown in soil contaminated with different concentrations of copper, zinc and lead

Studying the masses of strawberries at harvest, we observe from Figure 15 that, only in the case of soil contaminated with lead in 4.5% concentration, the mass of the fruits is maximum 30 g, at the other concentrations and metals analyzed the masses of the fruits were below 25 g, as had the control sample.

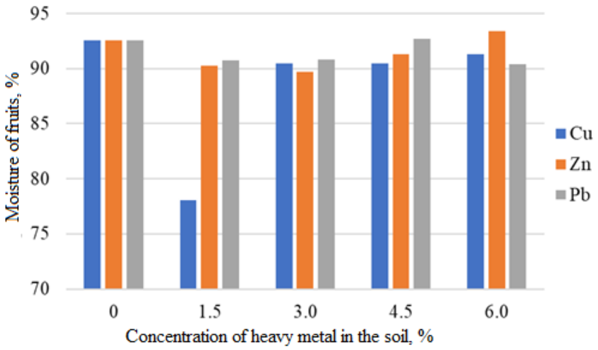

Fig. 16 – Moistures of strawberries grown in soils contaminated with different concentrations of copper, zinc and lead

The moisture of the strawberries grown in soils contaminated with copper, zinc, lead in different concentrations is approximately 92%, the value of the control sample.

3. Behavior of fruits and vegetables in the presence of heavy metals in the soil

The experiments of contamination with mixing of heavy metals carried out between March - October 2019, were done for 3 vegetables (carrot, parsley leaves, cucumbers) and 2 fruits (blueberries and strawberries).

All types of plants were cultivated in soil and contaminated with the following four heavy metal mixing concentrations (Cu+Zn+Pb): 1.5%, 3.0%, 4.5%, 6.0%.

In the experiments carried out with both vegetables and fruits, loading with heavy metal mixture was carried out only through the initial loading without further supplementation until harvesting.

The following are some aspects of the experimental research:

The stage of fruit development in soils with heavy metal mixture

The plants (vegetables and fruits) taken in the study were planted in a controlled environment using pots in which soil contaminated with four concentrations of Cu, Pb, Zn was added.

The solutions with concentrations of 1.5, 3.0, 4.5 and 6.0% were prepared individually using as reagent copper sulphate, lead acetate and Zn sulphate, the solvent used in the preparation of the solutions being distilled water.

For the mixing of Cu, Pb, Zn solutions for each of the 1.5, 3.0, 4.5 and 6.0% concentrations prepared individually, equal parts of each solution, element, respectively concentration were taken and mixed until homogenization resulted in the mixture.

The pots in which seedlings of vegetables and seedlings for blueberries and strawberry stumps were planted, were loaded with fertile soil that was mixed and homogenized in turn with each of the four solutions of different concentrations.

Compared to the soil contaminated with the four solutions of different concentrations, in parallel as reference samples, seedlings / stumps / stolons were planted in pots with uncontaminated fertile soil.

The determination of Cu, Pb, Zn from the contaminated soil and from the leaves and roots of the vegetables and from the studied fruits was performed by the *spectrophotometric method (atomic absorption in the flame).*

The variation of the heavy metal mixture content (Cu, Pb, Zn) in vegetables, at harvest, depending on the initial content of heavy metal in the soil, is graphically represented for carrots in Figure 17, for parsley leaves in Figure 18 and for cucumbers in Figure 19.

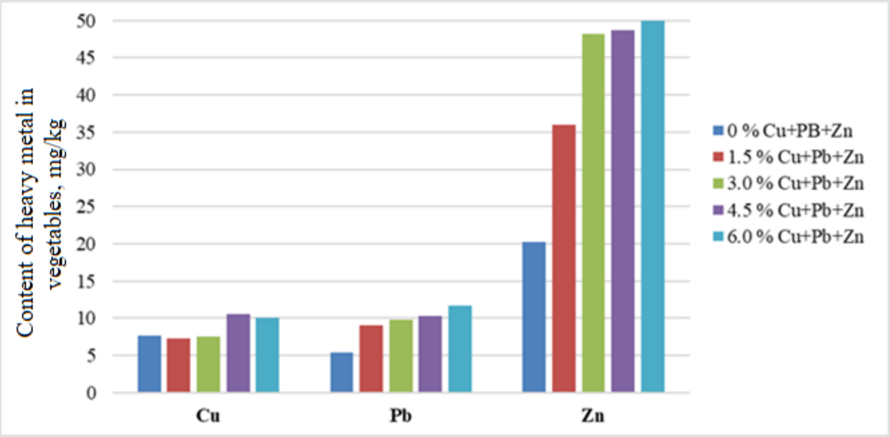

Fig. 17 - Variation of the Cu, Pb, Zn mixture content in carrots depending on the metal content in soil

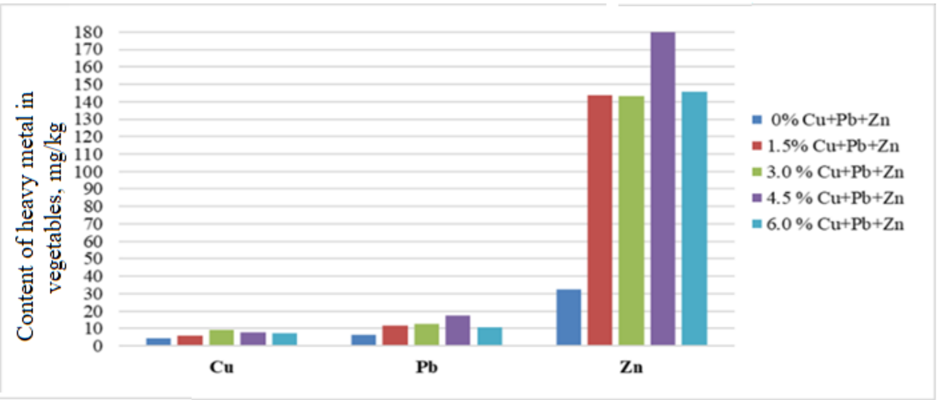

Fig. 18 - Variation of the Cu, Pb, Zn mixture content in parsley leaves depending on the metal content in soil

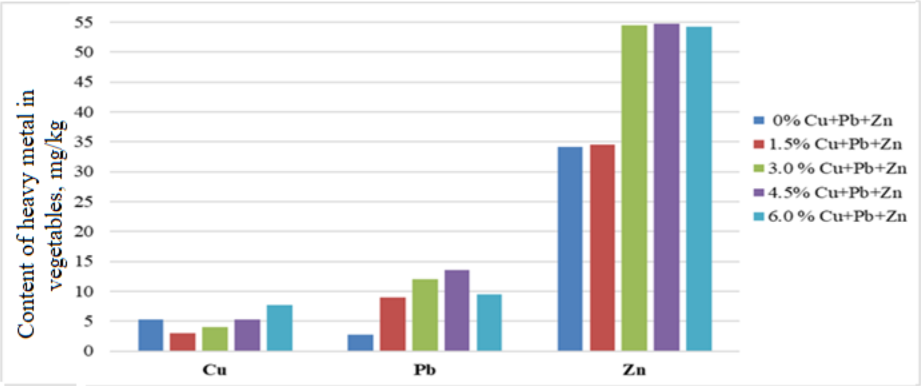

Fig. 19 - Variation of the Cu, Pb, Zn mixture content in cucumbers depending on the metal content in soil

The variation of the heavy metal mixture content (Cu, Pb, Zn) in fruits, at harvest, depending on the initial content of heavy metal in the soil, is represented graphically in Figure 20 for strawberries and in Figure 21 for blueberries.

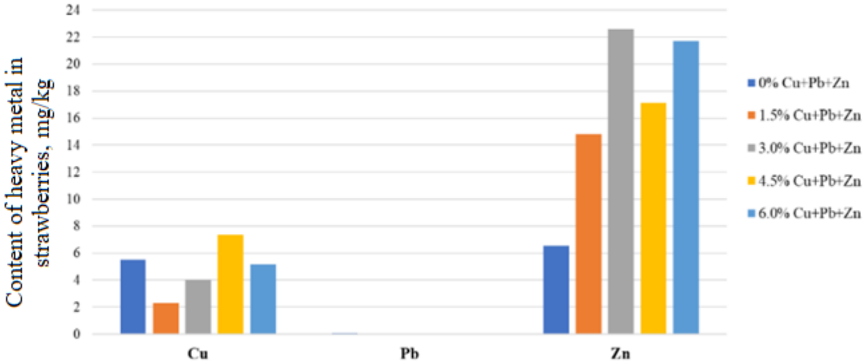

Fig. 20 - Variation of the Cu, Pb, Zn mixture content in strawberry fruits depending on the metal content in soil

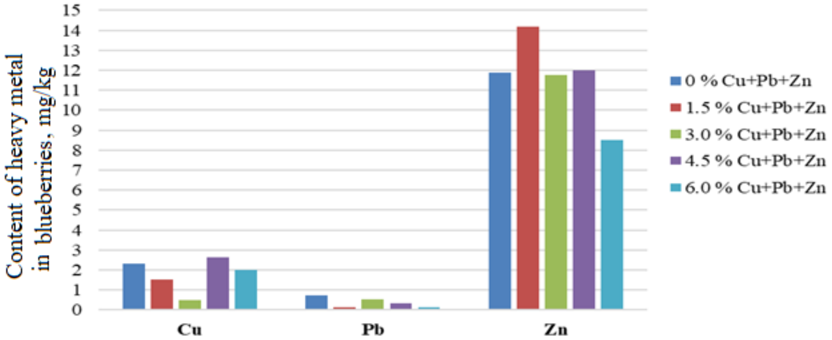

Fig. 21 - Variation of the Cu, Pb, Zn mixture content in blueberries depending on the metal content in soil

Considering all the cases of vegetables grown with different concentrations of heavy metals in the soil, it is observed that in the three vegetables: carrot, parsley and cucumber, there is a tendency to increase the amount of heavy metal accumulated in the plant, with the increase of the initial concentration of heavy metal in the soil.

In general, on vegetables, the conclusion regarding the increasing monotony of the curves representing the variation of the heavy metal concentration in the plant remains valid.

In the case of fruits: blueberries and strawberries there are some minimums and maximums, but we cannot comment because many repetitions are required. The minima and the maxima can represent accidents that can be avoided only by performing a large number of repetitions for the same experimental case.

The curves of variation of the concentration of heavy metal in plants that are not monotonous, appear in the case of contaminations with each of the three metals analyzed (copper, lead and zinc). In the case of strawberries the lead was undetectable by the working method used.

4. Experimental research on soil decontamination

In order to improve the contaminated soil, individual experiments were carried out for three methods, namely:

-The chemical method that consisted of adding EDTA in pots with soil contaminated with each of the metals: Cu, Pb, Zn;

- The biological method that consisted of planting mustard seedlings in pots with contaminated soil;
- Mixed method (chemical + biological) which consisted of planting mustard seedlings which were watered weekly with EDTA and water .

The choice of EDTA, from the multitude of chelating agents (EDDS, DTPA, HEDTA, citric acid) used in soil decontamination, was made after consulting numerous studies [6,7,11,12] on a rapid improvement method with substances, which showed the complexity of EDTA in the environment:

- can mobilize contaminating metal ions;
- can avoid precipitation of heavy metals in solution or, on the contrary, it may cause a dissolution effect of heavy metals adsorbed in sediments.

The results of the works [8,9,10,11,12] are based on the choice of the mixed method, regarding the rapid decontamination of soils using mustard and EDTA solution with different concentrations.

During the experiments were taken soil samples, respectively plant samples, to analyze the metals (Cu, Pb, Zn) from them. Each 4 mustard seedlings were planted in March in individual pots corresponding to the three metals and to the four concentrations related to each metal (1.5%, 3.0%, 4.5%, 6.0%) and weekly were watered without water (EDTA 0) and water in which 20 ml EDTA/weekly was added in three concentrations (EDTA 1 - 0.5 m, EDTA 2 - 1.0 m and EDTA 3 - 2.0 m). One month after planting from each pot, a mustard yarn was taken, which was measured and weighed, to observe / monitor the evolution of the plant one month after planting.

The soil used in the decontamination experiments was the soil used in the experiments on fruits and vegetables from the previous year (2018), phase 2 of the project.

Aspects during the experimental research, one month after planting, can be seen in Figure 22:

EDTA 0 EDTA 1 EDTA 2 EDTA 3

Plants grown in soil contaminated with Cu

EDTA 0 EDTA 1 EDTA 2 EDTA 3

Plants grown in soil contaminated with Zn

EDTA 0 EDTA 1 EDTA 2 EDTA 3

Plants grown in soil contaminated with Pb

EDTA 0 EDTA 1 EDTA 2 EDTA 3

Plants grown in soil contaminated with mixture of Cu, Zn, Pb

Fig. 22 – Mustard plants one month after planting

The following images show the mass (Fig. 23), height (Fig. 24), moisture (Fig. 25) and chlorophyll (Fig. 26) of the mustard plant at harvesting after one month of vegetation. The images shown are for the 3% concentration of each metal analyzed.

■ EDTA 0 ■ EDTA 1 ■ EDTA 2 ■ EDTA 3

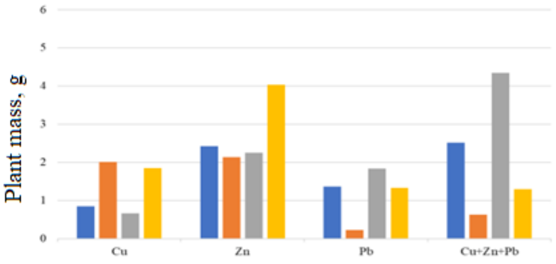

Fig. 23 – Plant mass

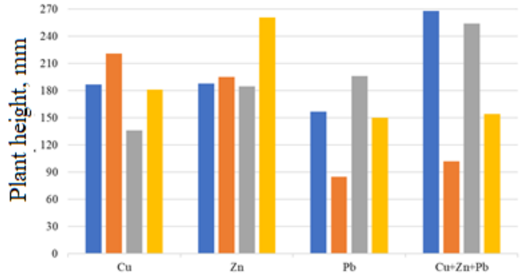

Fig. 24 – Plant height

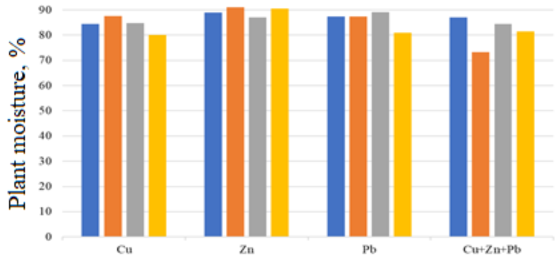

Fig. 25 – Plant moisture

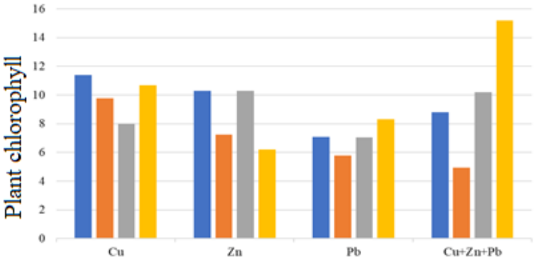

Fig. 26 – Plant chlorophyll

Parameters monitored one month after mustard planting

Regarding the mass of the plant, maximums of approx. 4.0 g in 2 cases, namely: for mustard grown in soil contaminated with Zn and treated with EDTA 3 in high concentration (2.0 m) and for mustard grown in soil contaminated with mixture of Cu+Zn+Pb and treated with EDTA 1 in concentration 0.05 m. The minimum mass of 0.2 g was recorded in the plant grown on soil contaminated with Pb and treated with EDTA 1 (0.05 m).

Plant heights ranged from 85 mm for plants grown in soil contaminated with Pb and treated with EDTA 1 (0.05 m), to 268 mm for plants grown in soil contaminated with a mixture of Cu + Zn + Pb and not treated with EDTA 0 .

Plants moistures varied between 73.3-91.9%. Chlorophyll content was reduced in plants grown in soil contaminated with EDTA addition at concentrations of 0.05 and 1.0 m, compared to the control sample, without addition of EDTA. Chlorophyll values ranged from 4.95 (Cu + Zn + Pb, EDTA 1) to 11.4 (Cu, EDTA 0) chlorophyll units. Chlorophyll values for mustard obtained in the experiment are lower than those obtained by the authors of the paper [5], where mustard has a higher content in chlorophyll.

The chlorophyll content was determined with the apparatus shown in Fig. 27.

Fig. 27 – Chlorophyll - meter

Aspects during the experimental researches, at the end of harvest, about 80 days, are seen in Figure 28:

Fig. 28 – Aspects during mustard vegetation

From the parameters monitored one month after planting the mustard, at the end of vegetation, about 80 days, only part of them were recorded, namely plant mass and plant moisture. In addition, the heavy metal content (mg/kg) of the plant was analyzed. These are shown in Figures 29 and 30.

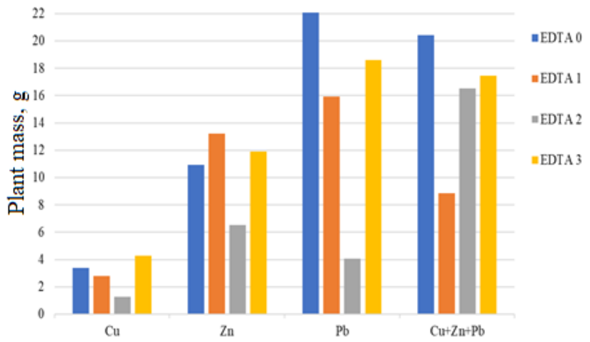

Fig. 29 - Plant mass at the end of vegetation

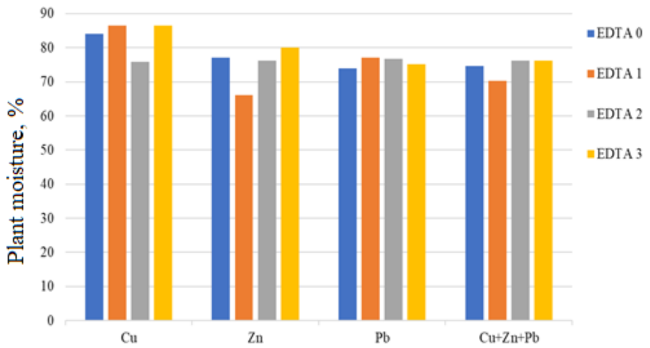

Fig. 30 – Plant moisture at the end of vegetation

Parameters monitored at the end of the vegetation

The masses of plants grown in pots with contaminated soil, at the end of the vegetation increased, as was naturally the case, less than those developed in soil contaminated with Cu, because at the middle of the vegetation period (40 days), the plants wilted. Plants grown in soil with Pb and metal mixture without addition of EDTA recorded the highest values of the masses: 22.08 g (Pb) and 20.42 (mixture).

Figures 31 and 32 show the metal contents in mustard grown in soil contaminated with each metal and in soil homogenized with a mixture of metals.

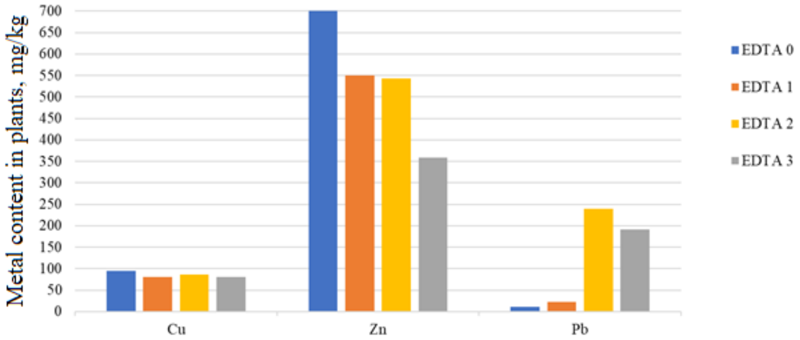

Fig. 31 - Metal content in plants

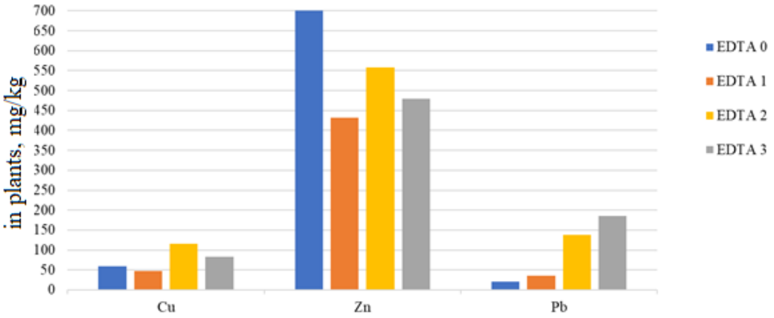

Fig. 32 - Mixture of metals content in plants

The metal content in plants grown in Cu and Zn soils shows a decrease in the metal content in the plant, as EDTA was added. In Pb soils, metal accumulation is higher when EDTA is added in high concentrations (1.0-2.0 m). In the case of the mixture we observe that the most absorbed metal by the plant is zinc, followed by Cu and Pb.

The plants reaching the end of vegetation had flowers, some also made seeds. These were weighed and the mass and moisture of each were determined (Table 1).

Table 1 – Masses and moistures of the mustard seeds that have reached maturity

Metal	EDTA concentration	Plant mass [g]	Moisture [%]
Zn	EDTA 0	1.1950	80.41
	EDTA 1 (0.05m)	2.4482	81.46
Pb	EDTA 0	2.0560	81.10
	EDTA 1 (0.05m)	2.2740	80.52
Mixture (Cu+Zn+Pb)	EDTA 0	1.5034	78.93
	EDTA 1 (0.05m)	2.1427	78.74
	EDTA 2 (1.0 m)	1.6262	82.64

Thus, it is observed that for copper, no plant has reached maturity or has flowered; those grown in soil contaminated with Zn, Pb and with a mixture of the three metals resisted those without the addition of EDTA and those with the addition of EDTA in very low concentration. In addition, plants grown in soil contaminated with a mixture of the three metals, have grown up to the seed stage and at a higher concentration of EDTA (1.0 m) added to the soil.

Below is the content of Cu and Zn from the initial soil (Table 2), soil recovered from the cultivation of vegetables and fruits from the previous year (2018). The soil was collected by metal and concentration categories, then homogenized well, samples were collected and the results are shown in Table 2.

Table 2 - The initial content of metals in the soils where the mustard seedlings were planted

Metal	Metal concentration, %	Metal content in soil, mg/kg
Cu	1.5	534
	3.0	1019
	4.5	1453
	6.0	1715
Zn	1.5	439
	3.0	654
	4.5	993
	6.0	1350
Pb	1.5	104
	3.0	151
	4.5	275
	6.0	318

Three decontamination methods were used:

1. *The phytoremediation method*. Soil in which mustard was planted;
2. *The chemical method.* Soil in which EDTA was applied weekly, each 20 ml EDTA in concentration 0. 5 m, 1.0 m and 2.0 m, for 60 days.
3. *The mixed method (phyto-chemical).* Soil in which mustard was planted, which was watered weekly for 60 days with 20 ml EDTA in concentration 0. 5 m, 1.0 m and 2.0 m;

It was aimed to identify an optimal method of improving the quality of contaminated soils.

Figure 33 shows the percentage content of metals in soils contaminated with 1.5% solution, Cu, Zn and Pb, and treated with EDTA in 3 concentrations (0.5m, 1.0 m, 2.0 m) trying a chemical remediation, compared to the soil in which only mustard (0 m) was planted, a phytoremediation with mustard was tried.

Figure 34 presents the percentage content of metals in soils contaminated with 1.5% solution, Cu, Zn and Pb, and treated with mustard planted as seedlings and EDTA in 3 concentrations (0.5m, 1.0 m, 2.0 m), trying it phytochemical remediation of the soil, compared to the soil where only mustard (0 m) was planted, trying a phytoremediation with mustard.

The percentage content of metals in soils contaminated with 1.5% solution, Cu, Zn and Pb, and treated by various methods was calculated as a ratio between the initial amount of metal in the soil and the amount of metal remaining in the soil after applying one of the three methods of soil decontamination.

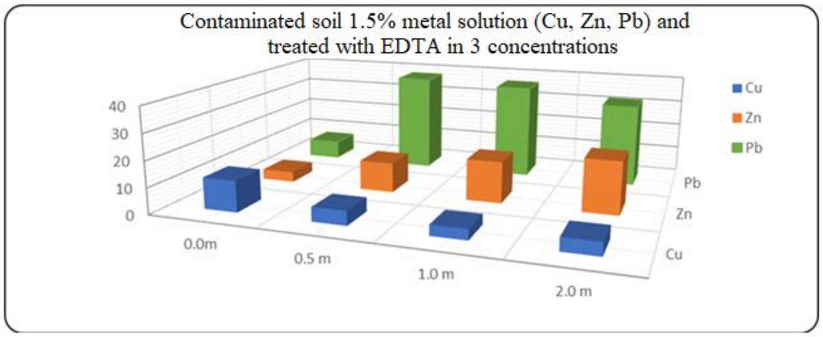

Fig. 33 - Percentage content of metals in soil contaminated and treated with mustard and EDTA in different concentrations

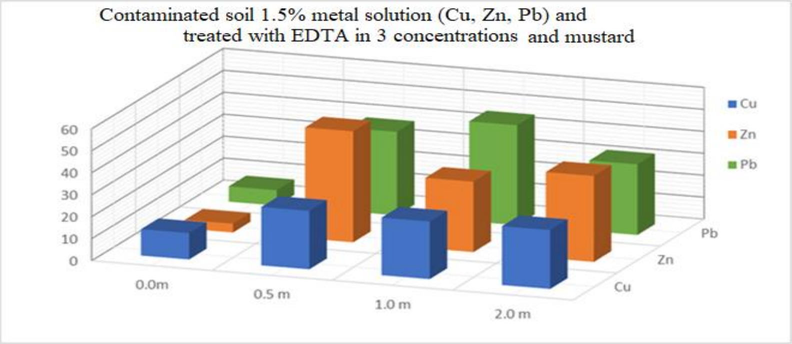

Fig. 34 - Percentage content of metals in soil contaminated and treated with mustard and mustard and EDTA in different concentrations

For soils contaminated with Cu, the phytoremediation method was effective (12% soil decontamination), compared to the EDTA method (below 5%), in contrast to the mixed mustard method and EDTA was more effective, about 26%.

For soils contaminated with Zn and Pb, the phytoremediation was weak (4% in the case of zinc and 7% in the case of lead), and the most effective method of decontamination proved to be all mixed (with mustard and EDTA), with content percentage greater than 32% for both zinc and lead.

Figure 35 shows the percentage content of metals in soils contaminated with 1.5% solution, Cu, Zn and Pb, and treated with EDTA in 3 concentrations (0.5m, 1.0 m, 2.0 m) trying a chemical remediation, compared to the soil in which only mustard (0 m) was planted, a phytoremediation with mustard was tried.

Figure 36 presents the percentage content of metals in soils contaminated with 1.5% solution, Cu, Zn and Pb, and treated with mustard planted in the form of seedlings and EDTA in 3 concentrations (0.5m, 1.0 m, 2.0 m), trying a phytochemical remediation of the soil, compared to the soil where only mustard (0 m) was planted, trying a phytoremediation with mustard.

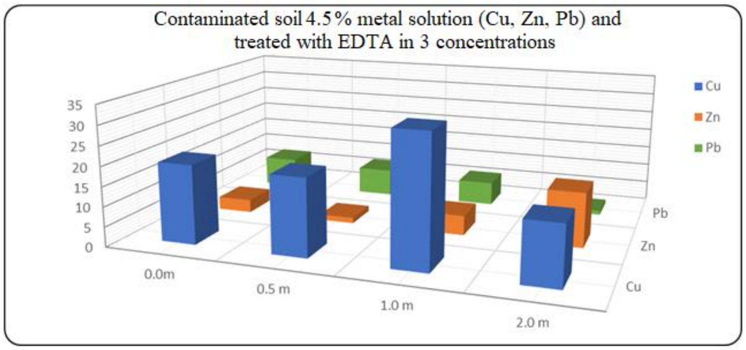

Fig. 35 - Percentage content of metals in soil contaminated and treated with mustard and EDTA in different concentrations

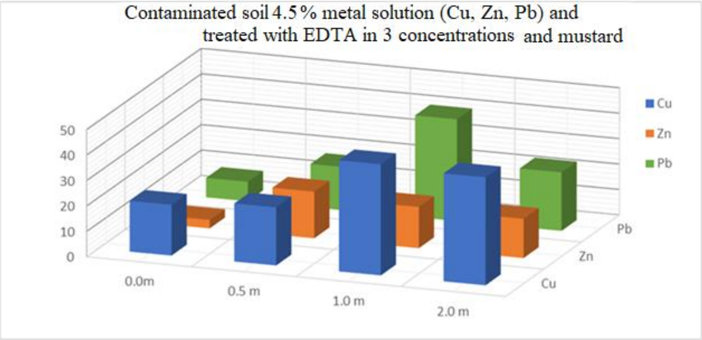

Fig. 36 - Percentage content of metals in soil contaminated and treated with mustard and mustard and EDTA, in different concentrations

The chemical method proved effective for each of the three metals, compared to phytoremediation, although at higher concentrations of contaminated soil, copper was extracted at most 20%.

In the case of the three metals, the mixed method (mustard with EDTA) had the maximum efficiency, compared with both chemical and the phytoremediation methods.

Experimental studies with mustard, mustard and EDTA, EDTA in different concentrations have shown that in time they lead to the decontamination of soil with Cu, Zn and Pb the most effective treatment, for decontamination of soils contaminated with the three heavy metals, irrespective of the degree of contamination, being planting mustard, watering during vegetation with water and EDTA solution.

5. Concluzii

Cateva concluzii generale despre ***variatia in timp a concentratiei metalului greu in salata*** cultivata in cele trei categorii de soluri, sunt evidente:

- globally, without altering the concentration of heavy metal in soil, the accumulation of heavy metal in plants increases;
- at the local level (for certain subintervals of time) the concentration of heavy metal may decrease in plants (obviously, some measurement or cultivation errors are abstractions) - this phenomenon is interesting because, if it is real, it may indicate the fact that the removal of heavy metal from the plant can be done naturally, remaining for future experiments to determine the influential factors in this process;
- the accumulation of heavy metal in plants grows from plants grown in the soil least contaminated with heavy metal to plants grown in soil with the highest initial hard metal concentration;
- for the soil contaminated with higher concentration of heavy metal (4.5%), the accumulation is more intense, at least for the range of values covered by these experiments;
- the masses of the plants that grow in the three types of soils, increase until the vicinity of the optimal vegetation period (45-50 days) [18], approximately monotonous, and then they still grow up to about 130.6% of the standard vegetation period, and further, without exception they decline;
- regarding the geometrical characteristics of the plants, the behavior of both (height and generalized diameter), varies quickly up to an average height (in about 25% of vegetation period), after which it varies in a narrow range around this value.
- the influence of the growth parameters (medium) on the evolution of mass (the recorded parameters: pH, plant moisture and soil moisture), at least in their (relatively small) variation intervals, is not obvious as it results from the calculation of correlations. Specifically, pH and moisture were maintained with as little variation around an average value.

Research on the accumulation of heavy metals in strawberries has revealed that the order of accumulation of metals in fruits (strawberries) is Zn> Pb> Cu. Copper is below the limit of the control sample, this would mean that with the high concentration increase the absorption capacity of the heavy metal in the soil decreases (for strawberries), which could lead to explanations related to the possibility of the plant to develop mechanisms for its growth. protection.

The masses of the strawberries were between 10 g (in the case of Cu, 1.5%) and 30 g (in the case of Pb, 4.5%).

Behavior of fruits and vegetables in the presence of the heavy metal mixture in soil - conclusions:

Considering all the cases of vegetables grown with different concentrations of heavy metals in the soil, it is observed that in the three vegetables: carrot, parsley and cucumber, there is a tendency to increase the amount of heavy metal accumulated in the plant, with the increase of the initial concentration of heavy metal in the soil.

Generally, on vegetables, the conclusion regarding the increasing monotony of the curves representing the variation of the heavy metal concentration in the plant remains valid.

The curves of variation of the concentration of heavy metal in plants that are not monotonous, appear in the case of contaminations with each of the three metals analyzed (copper, lead and zinc). In the case of strawberries the lead was undetectable by the working method used.

The experimental results obtained regarding soil decontamination show the behavior of the mustard during a vegetation cycle of the plant grown in soils contaminated with different heavy metals, the accumulation of metals from the soil contaminated in the mustard plants, soil that was treated with EDTA in 3 concentrations and as reference sample the soil contaminated with EDTA but without the addition of the chelating agent (EDTA).

The result of EDTA in the environment is an increased mobilization of heavy metals.

Research on the parameters monitored one month after planting the mustard:

- plants with small masses were recorded in those grown in soil with Cu (0.6-1.8 g) and with Pb (0.2-1.3 g);
- plants with large masses were recorded in those grown in soil with Zn (2.1-4.0 g) and mixture of Cu+Zn+Pb (1.3-4.3 g), except for the mustard grown in soil contaminated with Cu + Zn Pb mixture and treated with EDTA 1 (0.5 m);
- of the three heavy metals used, we conclude that Zn was beneficial for the growth and development of mustard plants, regardless of the concentration of EDTA added, the plants that did not grow were those grown without soil with Pb;
- the lowest heights were for plants grown in soil contaminated with Cu (136 mm) and Pb (85 mm);
- plants moistures varied between 73.3-91.9 %;
- the chlorophyll content was increased in plants grown in contaminated soil without the addition of EDTA and with the addition of EDTA 3 in high concentration 2.0 m.

Research on the parameters monitored at the end of the mustard vegetation period:

- at the end of the vegetation, the plants grown in soil with Pb and a mixture of metals (Cu, Zn, Pb) without addition of EDTA recorded the highest values of the masses: 22.08 g (Pb) and 20.42 (mixture);
- the mustard plants that developed until the flowering and fruiting stage in the form of silica with the seed were those for the metals Zn, Pb and the mixture of the three metals, Without the addition of EDTA and with very little addition (0.5 m) a EDTA; the plants grown in the soil with Cu, did not reach maturity, they were the first ones that disappeared in the middle of the vegetation period (30 days) and did not manage to develop until about 60 days as the other plants studied;
- metal content found in mustard plants shows a good absorption of zinc, followed by copper and finally lead.

Experimental studies on the decontamination of soils contaminated with the three heavy metals by the three methods: phytoremediation with mustard, remediation with EDTA and remediation with mustard and EDTA, in different concentrations, have shown that in time they lead to soils remediation contaminated with Cu, Zn and Pb. The most effective treatment, regardless of the degree of soil contamination, was the mixed one, that is planting mustard in the soil, watering during vegetation with water and EDTA solution.

Activity 3.2. – Validation of mathematical models

1. Statistical modeling of experimental data on the accumulation of heavy metals over time in green salad

The experimental data can be used to obtain interpolated variants of the functions of increasing the mass and geometrical dimensions of the plants, as well as for the functions that give the time variation of the heavy metal concentration in the plant. Starting from these interpolation functions, one can calculate other characteristics of the evolution process of plants grown in soil infested with heavy metals (in this case with Zn).

1.1. One-dimensional interpolations

Variation of plant mass

The variation of the mass of the plant samples collected in the temporal sequence, given in Fig. 37, it is interpolated with polynomial curves without a precise indication on the degree, indication provided for physical or biological reasons related to the growth phenomenon itself. Thus, the primary interpolation as a linear regression (1st degree polynomial), is given in Fig. 37. Experimental values are given in the same figure.

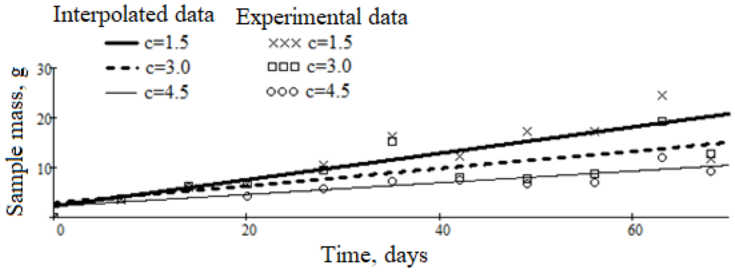

Fig. 37 - Linear interpolation of the growth of masses of variants of green salad crops in soil contaminated with heavy metal, Zn.

It can be observed the monotonous growth suggested by this interpolation, the natural tendency of the plants. In addition, it is observed that linear interpolation confirms the proportionality between the intensity of soil contamination and the mass growth of plants.

Variation of heavy metal concentration in the plant

With the same interpolation tools, similar results can be obtained about increasing the concentration of heavy metal in plants. Figure 41 shows the linear interpolation of the heavy metal concentration increase in plants, for each of the three variants of soil contamination. The experimental data are also represented. It is observed that in the more intensely contaminated soils, the accumulation of heavy metal in plants is more intense and faster (the slope of the corresponding rights is higher).

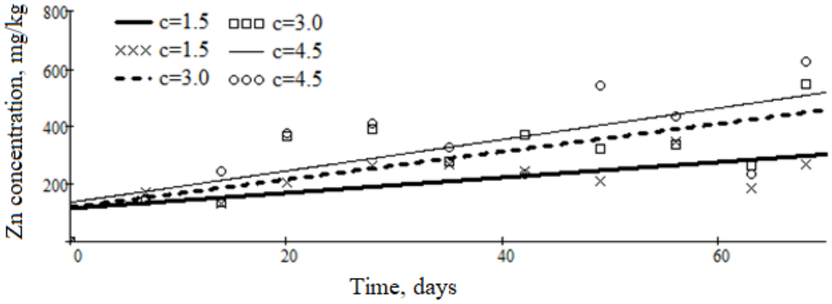

Fig. 41 - Linear interpolation of the increase of Zn concentration in the plant, corresponding to the variants of green salad crops in soil contaminated with heavy metal

In general, the order and monotony of the interpolation curves of the variation of the heavy metal concentration in plants is respected by the 4th degree polynomial curves (Fig. 44). Also, with very small exceptions (curve corresponding to soil contamination with 3.0% solution, it 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 in soil contaminated with solution having the concentration with 1.5%), the separation of the curves is well respected.

Plant height at harvesting

Plant height, measured from the soil at the time of harvest is a significant growth parameter. For this reason, like the mass or the diameter, it was recorded for each plant at each stage of harvesting.

Linear interpolation leads to the regression right lines in Fig. 45. Plant height is a positive characteristic of evolution and, as expected, the behavior is similar to that of the mass of the plant: the more the soil is more intensely contaminated, the higher is plant height at harvest.

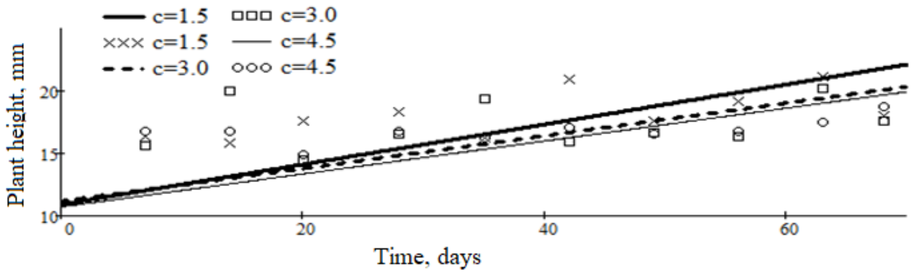

Fig. 45 - The regression right lines obtained by linear interpolation for the variation of plant height at harvest of the three crops, corresponding to the intensity of heavy metal contamination, comparative and comparative with the experimental data

Plant diameter at harvesting

In Fig. 49 are represented graphically the variations of plants diameter in time.

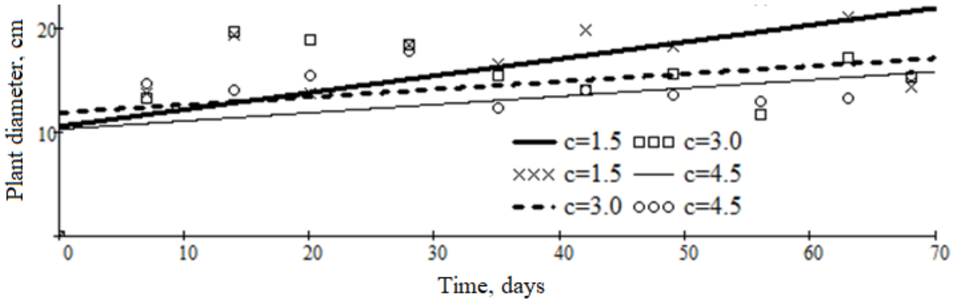

Fig. 49 - The regression right lines obtained by linear interpolation for the variation of plant diameter at harvest, corresponding to the three crops, after the intensity of heavy metal contamination, comparative and comparative with the experimental data

All the interpolations perform a mediation of the experimental data using the least squares method. It is observed that the increase of interpolation polynomial degree results in a better approximation of the experimental data, but we have no phenomenological reasons to grow above the 3rd degree of the interpolation polynomial. Another reason why we did not tried to increase the degree of interpolation 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. Hence, the statistical model can be used to validate the theoretical (dynamic) model, which is generalizable not only from the point of view of climatic conditions, but also of the types of plants used.

A number of recent works support the presented results, which guarantees our results: [13,14,15,16].

2. Interpolation of the experimental data in the mixture of metals in fruits and vegetables

$$Cp = \left(C_{Cup}, C_{Pbp}, C_{Znp}\right) \tag{4.1}$$

$$Cp = \left(C_{Cup}\left(C_{Cus}, C_{Pbs}, C_{Zns}\right), C_{Pbp}\left(C_{Cus}, C_{Pbs}, C_{Zns}\right), C_{Znp}\left(C_{Cus}, C_{Pbs}, C_{Zns}\right)\right) \tag{4.2}$$

The linear regression corresponding to this data set will take the form:

$$C_{Cup} = \alpha_{Cup} + \beta_{Cup} C_{Cus} + \gamma_{Cup} C_{Pbs} + \theta_{Cup} C_{Zns} \tag{4.3}$$

$$C_{Pbp} = \alpha_{Pbp} + \beta_{Pbp} C_{Cus} + \gamma_{Pbp} C_{Pbs} + \theta_{Pbp} C_{Zns} \tag{4.4}$$

$$C_{Znp} = \alpha_{Znp} + \beta_{Znp} C_{Cus} + \gamma_{Znp} C_{Pbs} + \theta_{Znp} C_{Zns} \tag{4.5}$$

Higher degree functions (polynomial regression or other types of functions) can be in case of multiple data and repetitions.

Table 3 - Interpolated data coefficients for fruits and vegetables

Fruits/ Vegetables	Coefficients of functions											
	α_{Cup}	β_{Cup}	γ_{Cup}	θ_{Cup}	α_{Pbp}	β_{Pbp}	γ_{Pbp}	θ_{Pbp}	α_{Znp}	β_{Znp}	γ_{Znp}	θ_{Znp}
Strawberry	2.417	0.034	-0.15	0.05	-3.887	0.15	-1.192	0.307	0.799	0.272	-1.657	0.389
Blueberry	-1.049	0.223	-0.989	0.156	0.907	$-8.756 \cdot 10^{-3}$	0.037	$-6.911 \cdot 10^{-3}$	11.529	0.015	-0.133	0.028
Carrot	7.723	$1.584 \cdot 10^{-3}$	0.039	$-8.306 \cdot 10^{-3}$	4.152	0.02	-0.135	0.045	7.636	0.61	-1.397	0.22
Parsley leaves	3.968	-0.037	0.053	0.02	4.816	-0.012	-0.094	0.06	$1.244 \cdot 10^{-3}$	0.452	-4.044	1.342
Cucumbers	5.665	$-1.429 \cdot 10^{-3}$	0.099	-0.029	0.953	-0.023	-0.104	0.076	33.278	-0.218	0.662	$4.48 \cdot 10^{-3}$

Because experimental data on fruits and vegetables grown in soils contaminated with metal mixtures do not have linear distributions, many of the values taken from outside the interpolation points have negative values, which was natural.

The linear function of 1st degree that could be taken into account does not have minima and maxima, and for the function of 2nd degree there were no longer sufficient data to determine the coefficients.

The small number of cases considered has as a consequence the impossibility to obtain nonlinear interpolations, at least 2nd degree polynomials, because we have three variables and therefore must be determined (for the complete polynomial of the 2nd degree in three variables) ten coefficients, we have only five experiments. Therefore, only linear regression is possible to obtain for which only four coefficients are required.

Although linear interpolation is not indicated, because we did not have nonlinear alternatives, we still studied linear regressions and observed that linear regression produces negative values over the experimental intervals that were worked, that is, negative values of heavy metal concentrations in straws, which did not have physical interpretation.

Interpolation curves for strawberry

Figures 54, 55, 56 show the interpolation curves for strawberries grown in soils contaminated with Cu, Zn and Pb in the four concentrations. It can be observed the parabolic and cubic interpolation curve, compared to the experimental data. The cubic curve for the zinc content of strawberry fruits is very close to the experimental data and the other curves for the copper content, respectively lead, are outside the experimental data.

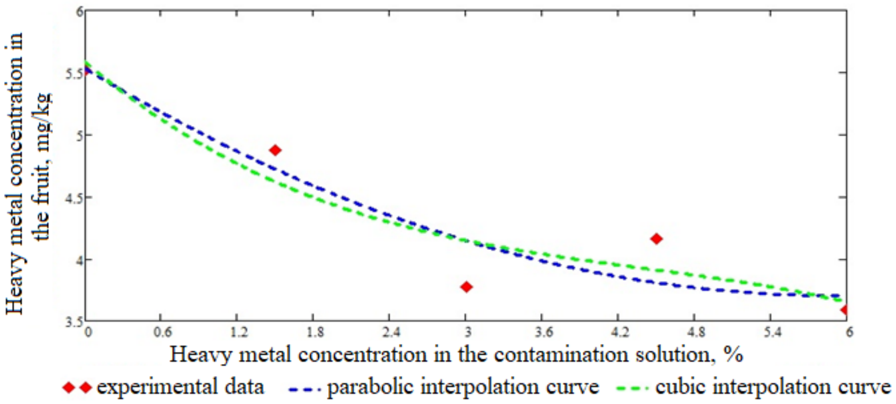

Fig. 54 - Graphical representations of the interpolations for copper content in strawberry fruits

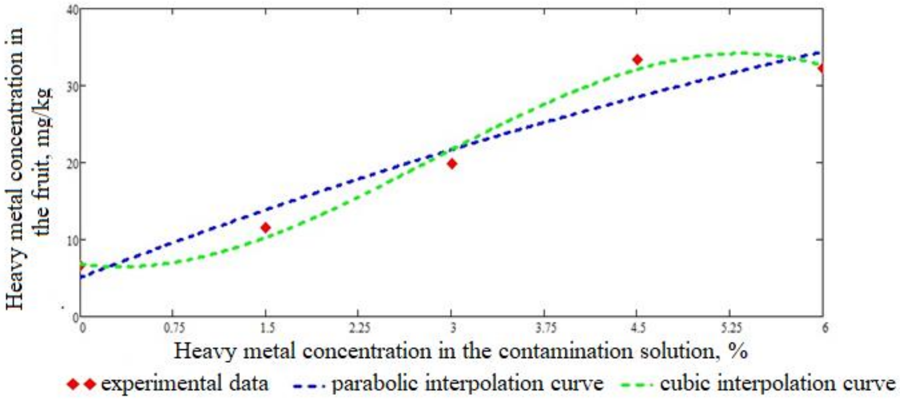

Fig. 55 - Graphical representations of the interpolations for zinc content in strawberry fruits

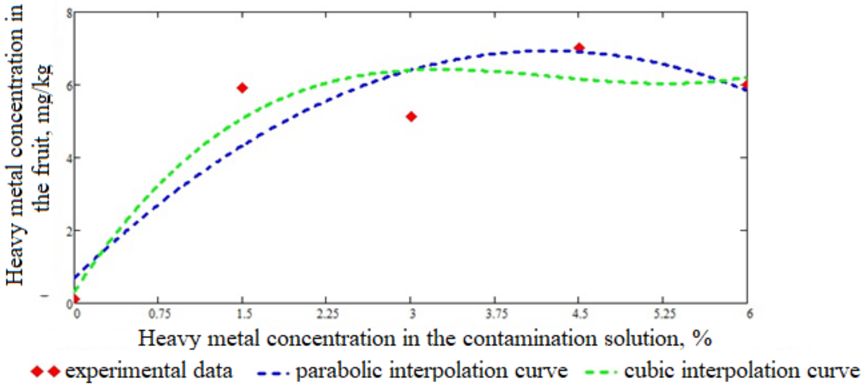

Fig. 56 - Graphical representations of the interpolations for lead content in strawberry fruits

3. Conclusions

Conclusions regarding the statistical modeling of experimental data regarding the accumulation of heavy metals in time in the green salad:

All the interpolations performed within the statistical models that are presented in this material, perform a mediation of experimental data. It is observed that the increase of the polynomial interpolation degree results in a better approximation of experimental data, but we have no phenomenological reasons for which to increase above the 3rd degree of the interpolation polynomial. Another reason why we are not tempted to increase the degree of interpolation 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 from the point of view of climatic conditions, but also of the types of plants used.

The variation in time of the growth of the mass of green salad plants cultivated in zinc contaminated soil confirms the result of the theoretical prediction made in paper [17]. Specifically, increasing the concentration of heavy metal in the soil leads to growth slowing, to the development of specimens of plants with a lower mass, height and diameter, also smaller than plants from the same lot, grown in soil not contaminated with zinc.

The results obtained by interpolating the experimental data and interpreting them are most likely to give very important indications for the next experimental step: the prolongation of the experiments over at least 2-3 generations of plants, which will allow to draw 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.

Conclusions -Interpolation of experimental data in the mixture of metals in fruits and vegetables

Because experimental data on fruits and vegetables grown in soils contaminated with metal mixtures do not have linear distributions, many of the values taken from outside the interpolation points have negative values, which was natural.

The linear function of 1st degree that could be taken into account does not have minima and maxima, and for the function of 2nd degree there were no longer sufficient data to determine the coefficients.

For blueberries and strawberries fruits, there appear some minimums and maximums, but we cannot comment because many repetitions are required. The minima and the maxima can represent accidents that can be avoided only by performing a large number of repetitions for the same experimental case.

Final conclusions - impact of the obtained results

The objectives of this project to develop original models based on the correlation between *the level of soil contamination* and *the remanence of pollution with substances in fruits and vegetables harvested for fresh consumption* and *the optimal method of reducing / improving / eliminating / dismantling / controlling the polluting substances in the soil* were achieved by the results obtained for fruits and vegetables, which shows that such experiences and statistical modeling can be the fundaments for building dynamic mathematical models that can simulate the life of a plant, even that of several generations of plants, and highlight the possible mechanisms of self-defense developed by plants, adaptations to the new environmental conditions / variables (climate change), possibilities of recovery in time of the qualities of some plants.

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