ECOTOXICOLOGY ASSESSMENT MODEL OF PLANT-SOIL COMPLEX TREATED WITH RADOMIR METAL INDUSTRIES WASTE WATER

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ABSTRACT

The industrial environmental "hot spots" create significant ecological hazards for terrestrial and aquatic ecosystems. Guidelines and legislation often refer to the total amount of contamination without to estimate the complex relationship between the environmental factors and the toxicant. In cases of suspicion for adverse effect on the environment bio-assessment can be used as a tool to detect the presence of hazardous chemicals. Bioassays with vascular plants are considered to be universal tools of identifying the combined effects of pollutants. The purpose of this article is to evaluate the toxicological effect of plant-soil complex treated with wastewater from Radomir Metal Industries ". The conclusion is that the sewage from the metallurgical pant "Radomir Metal" is used properly for irrigation of arable land. The question can be which kind of plants is suitable to be cultivated there. The effluent is seems toxic for the aquatic systems and has a slight negative impact on the soil breathing and germination of treated plants. Nevertheless, in the bioassay for all examined plants stimulus effect under the treatment with soil extract on the weight of roots and stems have been registered.

Keywords: *risk assessment, soil pollution, soil respiration, energy of germination, root growth;*

Introduction

Human activities all over the earth have increased environmental pollution by heavy metals in agricultural soil. Contamination with heavy metal is a major problem for crop quality, human health, and environmental quality. Most of the heavy metals are persistent in soil because of their immobile nature (5, 23). Chemical analysis are often insufficient to provide insight into the potential ecological risk, since they do not allow an evaluation of possible combined effects of the different contaminants mixed together, as well as to the their bioavailabilty. Guidelines and legislation often refer to total metal content in the soil, while they do not consider what proportion of that total amount may be biologically available to the organisms. Actually, environmental risks are related to the bioavailability of metals in soils. Therefore, bioassays which can mitigate these constraints are recommended for the assessment of ecological risks in soils or other matrices (8).

Contaminated sites pose significant environmental hazards for terrestrial and aquatic ecosystems. They are sources of pollution and may result in ecotoxicological effects. At severely contaminated sites acute effects occur, but the core problem lies in possible long-term chronic effects. Effects of toxicants at high concentrations that induce a high rate of acute mortality are observed easily, even in complex communities. The effects of toxicants at low concentrations that do not immediately result in acute mortality are much more difficult detectable. In such cases, communities are shaped not only by the effects of the toxicant but also to a great extend by other environmental factors. As a result, the complex relationship between a multitude of environmental factors and the composition of the community obscures the effects of the toxicants (25).

Ecotoxicological effects occur at all levels of the biological organization, from the molecular to the ecosystem level. Not only may certain organisms be affected, but the ecosystems as a whole in its function and structure (16). The purpose of this article is by using bioassay to produce ecotoxicological assessment of the soil complex treated with wastewater from "Radomir Metal Industries".

Materials and Methods

Contamination of the study region

According to the World Bank report (51), Pernik/Radomir with ferrous metallurgy and cement manufacturing are included in the acute industrial environmental "hot spots" in Bulgaria. In these areas, high airborne levels of dust, sulfur dioxide, and lead are common along with hydrogen sulfide, mercaptans, ammonia, hydrochloric acid, hydrogen fluoride, and other substances according to the particular size (36).

Radomir Metal Industries

Bulgarian metal casting plant Radomir Metals, previously Leko Ko, is based the south-western town of Radomir, Pernik district. The plant has been erected on 1 800 000 m^2 area and situated at about 50 кm south-west of the capital of the Republic of Bulgaria - Sofia, on the road Sofia-Pernik - Radomir - Kulata – Athens (**Coordinates: 42°31'10"N 22°59'12"E**). It has excellent infrastructure, connected with railway network and natural gas supplying. The plant has its own transformer electric power station directly connected to the National high voltage electric-power network. Environmental protection is one of the priorities of the company. The purification facilities fully meet the requirements of the European Standards for the quality of the air and water (37).

Overall, the environment and biodiversity in the municipality of Radomir are preserved, as there are no large industrial and agricultural pollutants, and no cross contamination. Indicative of relatively clean natural environment are air samples (**Table 1**), soil (**Table 2**) and water shown below.

TABLE 1

Data on air, Radomir Municipality (38)

Parameters	**Measured values, µg/ m³**	**Allowed Maximum Single Concentration, µg/ m³**
Dust	151	65
SO_2 (sulfur dioxide)	299	350
NO_2 (peroxide of nitrogen)	49	250
O_3 (ozone)	29	180
CO_2 (carbon dioxide)	4.9	12
H_2S (sulfur hydrogen)	0.001	0.005
NH_3 (ammonia)	0.011	0.25

TABLE 2

Soil samples from the region of study, Radomir Municipality (38)

Metals:	**Cu (Cooper)**	**Zn (Zinc)**	**Pb (Lead)**	**Cd (Cadmium)**	**As (Arsenic)**
Allowed values mg/kg	260	340	80	3	25
Measured values mg/kg	42	83	28	0.08	0

** There no information how much and from where the soil samples are taken, and are they cover the full region adequate.*

„Radomir Metal Industries" has a complex permit № 145-NO/2008 on within the scope of paragraph 2.2. Annex 4 of the Law on Environmental Protection. Has developed a policy for environment protection aimed at limiting the negative effects on the environment and prevent pollution of groundwater. Under the complex permit "Radomir Metal Industries has established annual emission standards presented in the table below (**Table 3**):

TABLE 3

Direct annual pollutant emission standards

Pollutants	Mixed stream wastewater (industrial and domestic-faecal) kg / year
Common nitrogen	709,4
Common phosphorous	77,45
Arsenic and its compounds	1,54
Cadmium and its compounds	0,15
Chromium and its compounds	1,54
Copper and its compounds	8,52
Mercury and its compounds	0,15
Nickel and its compounds	0,42
Lead and its compounds	1,54
Zinc and its compounds	15,02
Phenols	4,64
Common organic carbon	6954,5
Chlorides	-
Cyanides	0,57

For the good environmental status, importance has the functioning of purifying station for faecal-bit and effluents from industrial plants (mainly "Radomir Metal Industries, which owns the treatment plant). Purification is a mechanical, biological, as is done drying and stabilization of sludge. Receiver of purified water was the Struma River (II - Second category receiving water). Along the outlet wastewater to the water treatment point, canal water is intensively used for irrigation. Industrial waste water is discharged to the water purification plant through a channel running through arable land. In this regard, research in this work is done to establish whether there is compliance with established standards.

Characteristics of the test – objects

In general, the aim of bioassays are to determine the substance concentration or dilution, in which occurred 50% mortality or change in the relevant indicator in the test-organisms for determined time (LC_{50}). For the purpose of the study are conducted tests with objects from different vegetable and cereal crop in the relevant standards (26).

In assessing the toxicity of the effluent of "Radomir Metal Industries" are using the following indicators:

- Soil respiration;
- Energy of germination and germination (Eg and G);
- Length of root and stem (Lr, Ls);
- Absolute dry weight of root and stem (Wr, Ws);

Description of the conducted tests

Generally bioassays are used to evaluate the toxicity of wastewater, polluted air, soil, sediment, etc. or a particular pollutant using standard test organisms. The latter are exposed to different concentrations of the substance and report mortality or change in behavior or morphology and physiology of organisms. In determining the toxicity followed standard protocols in order to have comparability of results (26).

The conducted tests were performed with sewage and soil extract in various dilutions, as well as solutions of $ZnSO_4$ in some cases, because of perceived zinc content in soil over the marginal limit concentration.

Soil respiration

Soil contamination negatively affects the number and activity of populations of soil microorganisms. Contaminated soils breathe more intensively during the first 30-40 min at relatively low concentrations that do not cause mass extinction, but only stress in populations. For a long time (2 to 4 hours) and at higher concentrations the breathing decreased and completely stops. So in fixed period - the first 40 min after contamination with the toxic substance concentration, degree of variation in the intensity of breathing to breathing contaminated soils from the same soil type under the same other conditions depend on the type and concentration of the contaminant.

For the correctness of the results of the bioassay (real reflection of the soil condition), it is held several times in different seasons and times of the day.

The reduced soil respiration, reflecting deterioration of the populations of soil organisms, affects the functioning of the whole community.

Exposure time of the conducted test in the research is 1 hour.

Tests for determination the energy of germination (Eg), germination (G), length of root (Lr), and length of stem (Ls)

The seeds of each of the test plants (garden cress, radishes, wheat, maize and alfalfa) are flooded with water and stayed 24-hour. They are arranged in 50 identical grew at equal distances in large plates on two-layer filter paper moistened with 10 ml of distilled water (control); or soil extracts in different concentrations.

On the lid of the dish is placed one-ply paper that is wetted by 5 ml of distilled water or appropriate solutions. Filter paper and Petri dishes are pre-sterilized. Each variant and control is set at 6 reps., after exposure from 48 hours length and weight of sprouts in each dish are measure. The results for each variant and control are averaged and treated statistically as shown.

For the tests, in advance, soil extracts are prepared dissolving 5 g soil in 200 ml distilled water or effluent in the appropriate dilution. They are leaved on a shaker for 16 hours.

The variations of the experiments are as follows:

- ***control*** - distilled water,
- ***version 1*** - 30% effluent,
- ***version 2*** - 50% waste water;
- ***version 3*** - 70% effluent,
- ***version 4*** - 100% effluent.

Absolute dry weight of root (Wr) and stem (Ws)

Reporting absolute dry weight of samples dried 48 hours at 85 ° C and weighed 0,001 g on an analytical balance.

Assessment of water and soil contamination

Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) have been determinate for the test sewage. By definition, BOD is that quantity of oxygen necessary for the conduct of biochemical oxidation of organic matter in dissolved and colloidal suspended state for a specific time period. Biochemical oxygen demand or BOD is a chemical procedure for determining the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period. It is not a precise quantitative test, although it is widely used as an indication of the organic quality of water (6). It is most commonly expressed in milligrams of oxygen consumed per liter of sample during 5 days of incubation at 20°C and is often used as a robust surrogate of the degree of organic pollution of water. As with BOD can not take into account the total mass of organic substances contained in water to obtain a more accurate and more complete assessment of the quality of organic matter, it is necessary to determine COD. In environmental chemistry, the chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers), making COD a useful measure of water quality. It is expressed in milligrams per liter (mg/l), which indicates the mass of oxygen consumed per liter of solution. Older references may express the units as parts per million (ppm).

Other indicators of water and soil, e.g. mechanical composition, content of elements in soil and wastewater, etc. were examined. The use of other methods:

- *Method of determining the oxidation;*
- *Method for determination of sulphate ions.*

The oxidation was determined in the Executive Environment Agency (EEA), Lab - Water and in the Faculty of Biology - Department of Hydrobiology, Sofia University "St. St. Kliment Ohridski".

Other methods such as Kaczynski method for determining mechanical composition of soil, acidity of the soil solution, humus content, and etc. characteristics were used. Soil samples were processed in Research Institute of Soil Science and Agroecology "N. Pushkarov" - Laboratory of Soil Genesis. The nitrogen content was studied in the Faculty of Biology, Sofia University "St. St. Kliment Ohridski", Laboratory for analysing amino acids. The study of bioaccumulation of heavy metals and others is carry on by using atomic-adsorption method. Samples were processed and measured in the Faculty of Chemistry, Sofia University "St. St. Kliment Ohridski".

Statistics

All obtained results were statistically processed. It has been shown that the resulting averages are representative of performance using t-test. The discussed values are average of 6 repetitions of each version and for 300 seeds or plants of option (50 pcs. for repeat X 6 reps). The statistical significance level in this study was defined at $p < 0.05$.

Results and Discussion

<u>Results of samples from wastewater</u>

The table below (**Table 4**) presents the results of some additional analysis of samples of waste water and limit concentrations of metals under Regulation № 6.

TABLE 4

Values of the analyzed parameters of samples effluent

Elements	Marginal limit concentrations*, $\mu g\ l^{-1}$	Acidified sample, unfiltered, $\mu g\ l^{-1}$	Not acidified sample, unfiltered, $\mu g\ l^{-1}$
Cd	500	3.2±0.2	2.3±0.2
Cu	500	12±2	8±1
Cr	500	2.8±0.2	2.1±0.1
Fe	5000	189±14	156±12
Ni	500	4±1	3±1
Pb	200	3.8±0.1	2.1±0.1
Zn	2000	230±15	190±12
Hg	10	<1	<1
Other indicators	pH – 7.92 Chemical oxygen demand (COD) (BBM 0208:2001; $t = 20^0 \pm 3$) - 17,2±1,63 COD (oximetric) 20,48 $mg.l^{-1}$ – filtered sample; 73,10 $mg.l^{-1}$ – unfiltered sample	NH_4^+ - 0.2mg/l NO_3^- - 5.7 mg/l	SO_4^{2-} - 19.5 mg/l Cl – 10 mg/l PO_4^{3-} - < 0.1 mg/l

The marginal limit rate are defined in the particular circumstances of production taken into account for the manufacture of iron and steel, production of iron and steel castings, cast cars and other non-ferrous metals.

The results show that the resulting concentrations are within the limits of regulation.

<u>Results of soil analysis used in testing</u>

The results of the analysis of soil used in the tests are presented in **Table. 5**. The study of the mechanical structure shows that the soil is sandy - loam average (4.5) and secured with average humus. Humus content is 5 %.

TABLE 5

Values of the analyzed indicators of soil samples

Elements	Samples	Other characteristics				
Cd, µg g^{-1}	1.2±0.2	Common nitrogen Keldal, gr %	pH	Bulk density, Q	Marginal field humid capacity, %	Humidity, W, %
Cu, µg g^{-1}	32±2	1.208	pH $_{H2O}$ 7.43	1.52	24	60.72
Cr, µg g^{-1}	48±3	1. 569	pH$_{CaCl2}$ 6.57			
Ni, µg g^{-1}	11.5±1.3	1.500				
Pb, µg g^{-1}	28±2	1.444				
Zn, µg g^{-1}	*147±25*	Average				
As, µg g^{-1}	12±3	1.430				

The comparison with the limit concentrations (down in the Ordinance on Soil Protection for use of sludge from waste water for agricultural purposes, **Table 6**), shows that they are in compliance. The only exception is the level of zinc, which exceed the maximum concentration and at the highest levels of pH.

TABLE 6

Limit concentrations of heavy metals in soil*

№	pH*1	Limits (MAC) mg / kg dry matter							
		lead	copper*	zinc	cadmium	nickel*	chrome	mercury	arsenic
1.	4	25	20	30	0.4	25	150	1	25
2.	5	40	40	60	0.8	35	170	1	25
3.	5.5	50	60	60	1	50	180	1	25
4.	6	70	120	200	1.5	60	190	1	25
5.	7 и >7	80	140	300	3	70	200	1	25

* The competent authorities may allow exceeding of those values with soil pH, lasting more than 7. The maximum limit concentration of these heavy metals must not exceed the values for pH = 7 with more than 50%.

Results of soil respiration

Soil respiration consists of autotrophic root respiration and heterotrophic respiration which is associated with decomposition of litter, roots and soil organic matter (SOM) (2). Soil respiration (CO_2 efflux), a common measure of soil health (12, 20, 21), and responds to contaminants but also to temporal changes in moisture, temperature, light conditions, and spatial variation in soil fertility (13, 14, 3). Positive exponential relationships between soil respiration and soil temperature, as well as positive linear relationships between soil respiration and soil moisture have been found in some warm and moist forests (45, 7, 47, 53, 31, and 32). It has been accepted that under the same hydrothermal conditions soil respiration would be influenced by the degree of contamination and can be used as a test for assessment of soil contamination.

The results of the bioassay showed that all tested concentrations lead to a change in the intensity of breathing of soil and affect the functioning of the communities. The impact with the lowest test concentration (30% effluent) leads to a strong reduction of soil respiration by 66,70% less intensity compare to control breathing (Fig.1). Changes in soil respiration by 50% compared to control (LC_{50}) may occur between 40-50% dilutions of effluent. Increasing the concentration causes stress reactions in populations of soil microorganisms associated with increasing intensity of respiration (**Fig.1**).

It has been reported by **Fliessbach & all**. (1994) similar results of increasing soil respiration and especially the respiration per unit biomass (qCO_2) with increasing amounts of heavy metals from contaminated sludge (17). A moderately contaminated sludge was applied as received from the sewage treatment plant and after additional metal contamination. The ratio of biomass C to soil organic C (Cmic/Corg) even decreased when low metal sludge was applied (17).

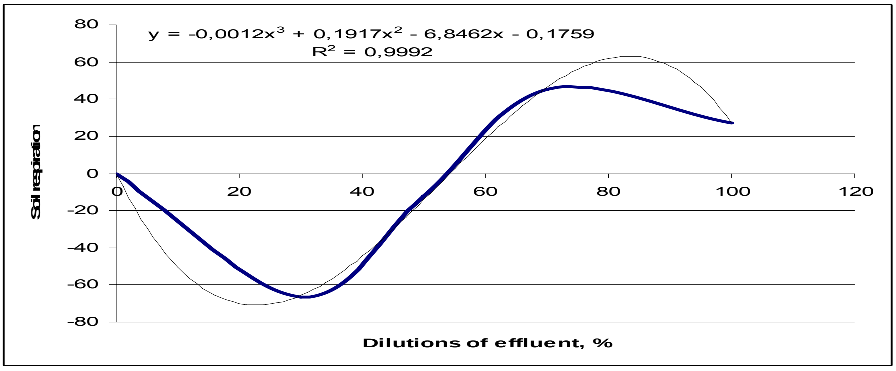


Fig.1 Alteration of soil respiration at soil moisture with a wastewater tested concentrations

In addition to concentration effects, most contaminants interact with the soil physiochemical structure to varying extents to impart additional variability (10, 49). For instance, the toxicity of heavy metals depends on soil acidity and organic matter because these factors strongly influence metal/metalloid bioavailability (27). As a result of these complex interactions, bivariate scattergrams of soil respiration values versus contaminant concentrations often display a characteristic 'wedge-shape' pattern that suggests contaminants act to limit maximum respiration values (48). The same dose-response curve is obtained when the influence of Ni-solutions with different concentration has been evaluated (26).

The results from study characterizing modifications in the genetic structures of soil bacterial communities induced by different doses of Cu, Cd, and Hg added independently or in combination (Cu + Cd + Hg), allowed authors to deduce the following order of impact: (Cu + Cd + Hg) >> Hg > Cd >Cu. The obtained results demonstrated that there was a cumulative effect of metal toxicity (41). Furthermore, the trend of modifications was consistent with the "hump-backed" relationships between biological diversity and disturbance (19).

According to the analysis of contaminated soil and wastewater zinc exceed the allowed limits of content that was the reason to evaluate the changes in soil respiration under the treatment with solutions of different concentrations of Zn ($ZnSO_4$). The registered tendency was that for the incubation period, soil respiration increased in comparison to control (**Fig. 2**). The most intensive respiration (30,80 mg CO_2) was registered at the lowest concentration (0,1mg/l), compare to control (3,15 mg CO_2), it is 879,02% higher. With increasing the concentration of zinc ions the intensity of soil breathing was reduced, but it remains much higher than the control, at least 279,72% over control in highest concentration (2,5 mg/l).

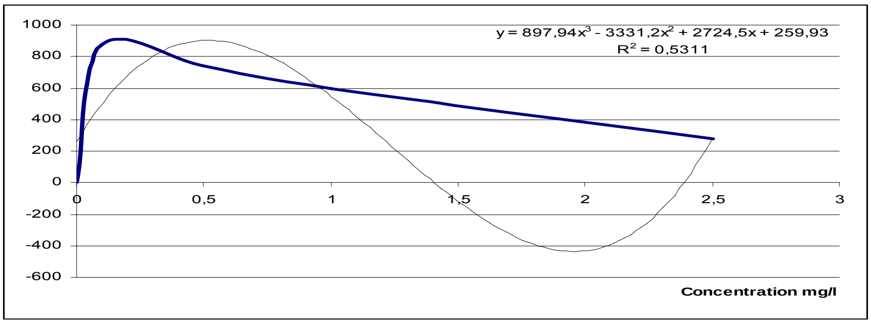


Fig. 3 Alteration of soil respiration under the effect of zinc ions

In a short-term laboratory experiment to evaluate the impact on soil microorganisms of different metals (Cu, Zn, and Cd) added singly or in combination was found that soil respiration and microbial biomass were significantly affected by Zn and Cu, respectively (43). In all cases, polymetal contamination had a greater impact than single-metal contamination, leading to conclusion that there were additive effects of metal toxicity (42).

Results from the tests for determination the energy of germination (Eg), germination (G); length of roots (Lr) and stems (Ls); and weight of roots (Wr) and stems (Ws).

Alteration the energy of germination (Eg), germination (G)

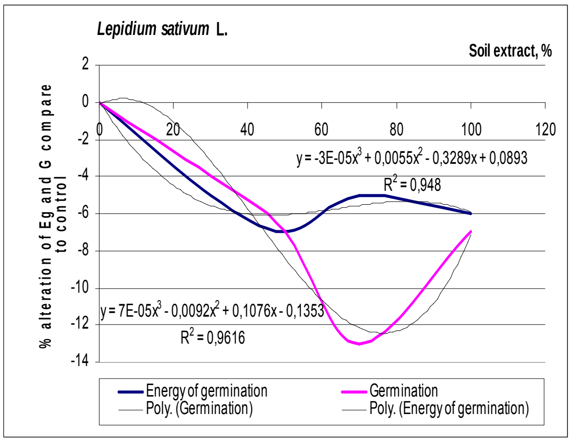
Bioassays with vascular plants are considered to be universal tools of identifying the effects of pollutants present in soils. Bio-assessment can be used as a tool to detect the presence of hazardous chemicals in the environment evaluating the effects of mixtures with the combined effects that can be expressed as synergism, additivity and antagonism demonstrating bioavailability of contaminants to different species (28, 35, 4, 24). The seed germination and early-seedling growth are important stages in the whole process of plant growth and due to being the most sensitive stage in the plants changing of their environment, have been widely used in environmental bio-monitoring. In order to make an ecotoxicological assessment of soil complex treated with sewage from the heavy metal plant the alteration of energy of germination *(Eg)* and germination *(G)* under the influence of soil extract on the seeds of different plant species has been examined (**Fig. 4)**.

Recently, demands have increased on assessment of ecological risks associated with soil contamination. To meet such demands, various higher plant bioassays have been developed for estimating potential effects of chemicals in soils, and for comparison of soils of known and unknown qualities. *Lepidium sativum* L. (cress), respectively are included in the OECD list of species used for toxicity assessment (35). The results of conducted bioassay showed that the energy of germination (*Eg*) of treated seeds of *Lepidium sativum* L. was lower from 5 to 7% compared to control with significance $p<0.05$. Most strongly inhibited energy of germination for garden cress seeds was at impact of 50% soil extract (**fig.4, a**). The germination *(G)* of *Lepidium sativum* L. under treatment with soil extract was lower from 4 to 13% compared to control seeds ($p<0.05$). Most suppression occurred in 70% extract (**fig.4, a**).

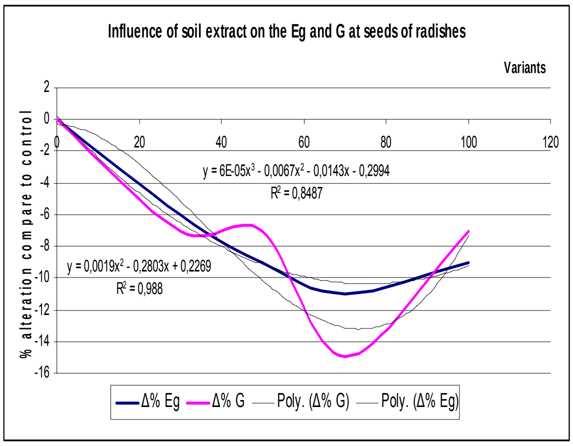
The process of reducing the energy of germination (*Eg*) for the seeds of *Raphanus sativus var. radicula* was by 6 to 11% compared to the control respectively in soil extract at 30% and 70% ($p<0.01$). The reducing of *Eg* was by 9% in the other 2 variants – 50% and 100% soil extract from the contaminated soil (**fig. 4, b**).

Germination decreased from 7 to 15% over control at 70% soil extract ($p< 0.01$) and 100% solutions germination of sprouting is relatively uniform (**fig. 4, b**). Both observed patterns for alteration of energy of germination *(Eg)* and germination *(G)* under the influence of soil extract on the seeds of *Lepidium sativum* L. and *Raphanus sativus var. radicula* were very similar (**fig. 4**, **a** and **b**). Germination and early seedling development assay has been regarded as a basic experiment for evaluating the toxicity effect of any kind of metal or chemical on plants (1, 29, 50). Germination inhibition is among the best-known effects of toxic impact of heavy metals (15). Under heavy metal stress, the processes of germination, like embryo growth, will be depressed (1). Some researchers (39) have reported the reduction of germination rate and seedling growth of different crops by heavy metals toxicity.

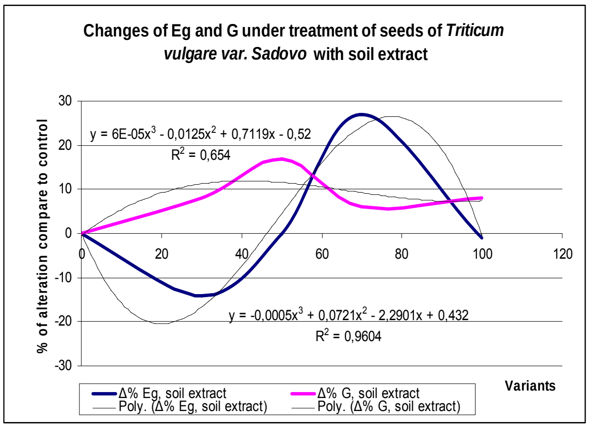
For the wheat seeds (*Triticum vulgare var.*Sadovo) reduction of *Eg* in variant-30% by 14% compared to control and stimulation effect by 27% and at variant-70% has been registered ($p< 0.01$) (**fig. 4, c**).



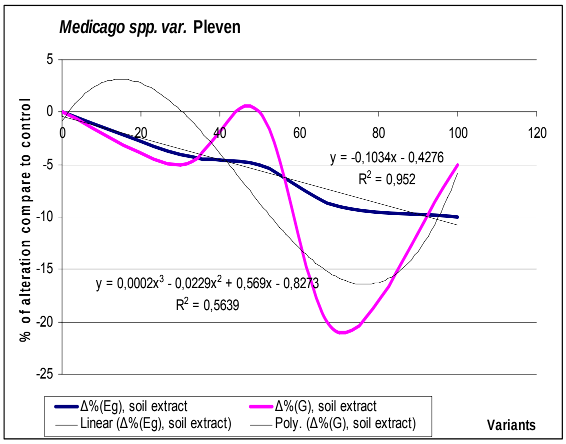
a) Influence of the soil extract on germination of seeds of *Lepidium sativum* L;



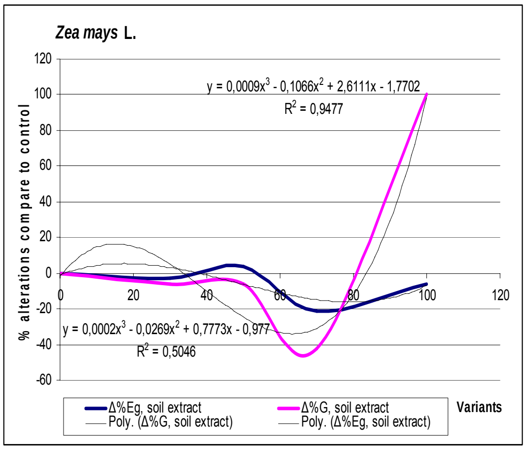
b) Influence of the soil extract on germination of seeds of *Raphanus sativus var. Radicula;*



c) Influence of the soil extract on germination of seeds of *Triticum vulgare var.Sadovo;*



d) Influence of the soil extract on germination of seeds of alfalfa (*Medicago* spp. L. *var. Pleven*);



e) Influence of the soil extract on germination of seeds of *Zea mays* L.

Fig. 4 Alteration of energy of generation (Eg) and germination (G) of the seeds under the influence of soil extract in different dilutions.

The influence of undiluted extract and variant-50% backs were weak. On impact with the soil extract was observed stimulation of germination of seeds, mostly pronounced at a dilution of 50% (**fig. 4, c**). Plants expressed different reactions to the metal soil contamination. When phytotoxicity tests, was carried out different responses of the plant species to sediment samples ranging from growth inhibition to growth stimulation has been reported (9). Heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, and Zn are plant essential metals and most plants perform stimulation effect when they present in the soil (22). Zinc is one of the necessary trace elements for plants and rural soils have optimum Zn content for plant growth. It has been indicated that the application of Zn can efficiently increase the yield of the *Thlaspi caerulescens* (44). In the sewage Zn is the metal that exceed the allowed limits of regulation (**Table 6**), also in the soil content it has higher level compare to other metals (**Table 5**). It has been reported that the application of Zn in the normal area and Zn deficient areas was effective in reducing the Cd concentration in the wheat grain. When growing in nutrient solution contained low concentration of Cd, a strong antagonistic effect of Zn on Cd accumulation was found in young leaves of lettuce or spinach (30).

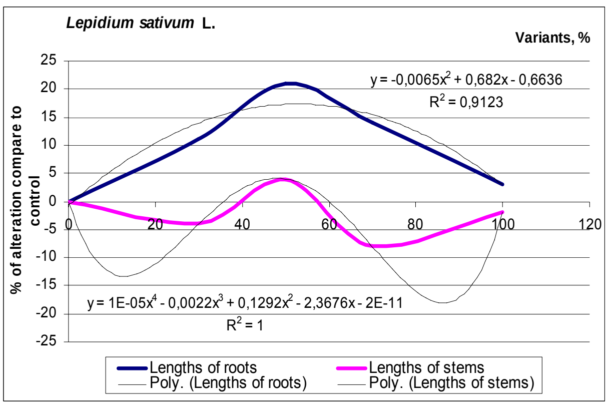
Clear and strong negative impact of soil extracts on *Eg* has been registered for alfalfa (*Medicago* spp. L. *var. Pleven*) seeds. The values of energy of germination were from 4 to 10% lower compare to control ($p<0.01$). With increasing the concentration, the negative deviation from the control sample increased (**fig. 4, d**). The linear regression with correlation R^2=0,952 has been obtained. There was registered also a negative influence on germination of seeds of alfalfa (*Medicago* spp. L. *var. Pleven*) from 5 to 21% compared to control at impact with the soil extract with significance $p<0.01$ (**fig. 4**, **d**). Mostly negative impact was observed in 70% extract.

On the germination of *Zea mays* L seeds, soil extracts associated with reduced jointly germination in treated seeds. Energy of germination decreased by 3 to 21% in treated seeds compared to control. Weak stimulation in *Eg* was observed under the influence of 50% soil extract (**Fig. 4, e**). There were reductions of germination from 6 to 42% on impact with the soil extract, version-3 (70% soil extract). Both indicators are with most strongly effects of variant-3 (70% soil extract).

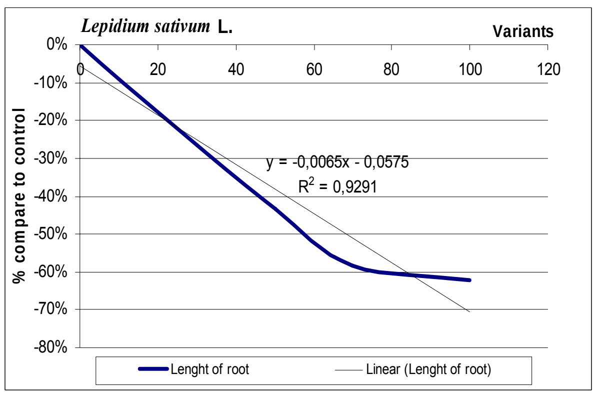
Length of root (Lr), and length of stem (Ls)

The toxicological effects of soil extract in different concentrations on the early development of the garden cress (*Lepidium sativum* L) were studied in measuring the lengths of stems and roots of treated plants. The resulting data showed that soil extract stimulate roots with 3 to 21% compared to control (**fig. 5, a**). This trend is less pronounced in the undiluted extract and most at 50% diluted. The reaction of the stems showed slightly retention of growth with 2 to 8% versus control, respectively undiluted extract and that with 70% soil extract. At 50% dilution was observed stimulation of growth by 4% over the control (**fig 5, a**).

Clear and strong negative effect on the growth of garden cress roots has been registered for the treatments with wastewater, **figure. 5, b**. The obtained results generate a linear regression with correlation coefficient R^2=0,9291 (**fig.8, b**). In that case can be seen retardation in the development of root from 26 to 62% compared to control, which increases with decreasing the dilution of sewage ($p<0.001$).



a) Influence of soil on *Lepidium sativum* L;



b) Influence of waste water on the length of root (Lr) of *Lepidium sativum* L;

Fig. 5 Influence of soil extract on the lengths of roots (Lr) and stems (Ls) of *Lepidium sativum* L.

The toxicity of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn ions were examined in blank, nitrate ($N–NO_3$)–, phosphate (KH_2PO_4)–, and saline (NaCl)–contaminated media. The acute toxicity of the tested metal ions in the blank media according to their IC_{50} (50% inhibitory concentration) values increased on the order of Pb<Fe<Co<Zn<Mn<Cr<Cu<Cd<Ni (34). Toxicity of single metals according to the relative growth of seedlings' root of *Lepidium sativum* L was revealed that the toxicity of the test metals to *Lepidium sativum* L decreased in the following order: Cr(VI) > Cu(II) > Ni(II) > Zn(II) (33). It was found that the impact of metals on terrestrial plant *L. sativum* and the interactions of metals within their mixtures differed. So, forecasting the changes in the functioning of the ecosystem under exposure of various contaminants it is necessary not only to determine the biological impact caused by multicomponent mixtures, made in accordance with naturally found concentrations, but also to assess the joint effects in these mixtures (33).

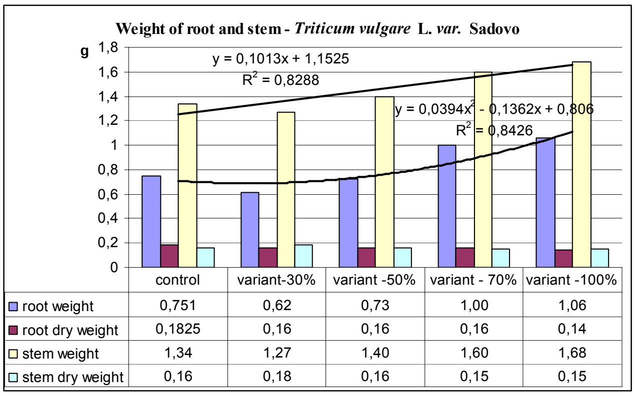
The results from of samples of waste water showed that resulting concentrations of metals are within the limits of regulation № 6 (**Table 4**). Many chemical mixtures, where concentrations of individual chemicals commonly exist at levels not considered toxic, are often present in aquatic systems. However, it is reckoned that chemical mixtures where individual constituents are present at low, non-toxic concentrations may trigger toxicity due to additive or synergistic effects among the constituents (40, 33). The antagonistic or additive interactive effects found in almost all metal ion mixture combinations confirms the presumption that the interaction between ions can be caused by competition for the same reaction center on cell membranes if these ions belong to the same group of Lewis acids (33).

In the case of combinations of Ni(II) + Cr(VI) and Cu(II) + Ni(II) at equal concentrations of each metal (5; 10 and 20 mg/l) the suppression of the inhibitory effects of Cr(VI) and Cu(II) was revealed. This allowed making a presumption that Ni may suppress the inhibitory effects of the above-mentioned metals to the root growth of *L. sativum*. Sresty and Madhava Rao (46) states that the inhibitory effects of Cu(II) and Cr(VI) may decrease due to Zn(II) and Ni(II); whose relatively low concentrations may induce a greater degree of plant cell vacuolization, increasing cell ability to reduce the cytotoxic effects of the metals. However, some authors propose that scilicet copper inhibited the binding and cellular uptake of zinc, which resulted in decreased toxicity of these metal mixtures to plants (18, 11**)**.

Weight of root and stem

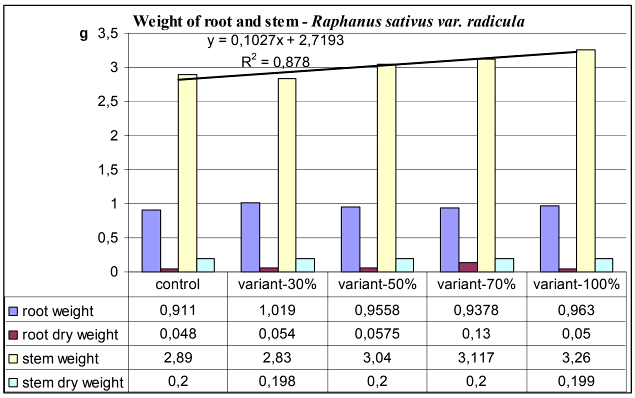
The results obtained for the influence of soil extract on the weight of root and stem of wheat sprouts are presented on **fig.6** (**a**). Overall, the impact of soil extracts on wheat (*Triticum vulgare* L. *var*.Sadovo) stimulates the gain of weight of roots and stems. The linear regression describe the trend for the weight of stems with correlation coefficient $R^2 = 0.8288$ (**fig.6**, **a**). Increasing the weight of roots have polynomial trend with $R^2 = 0.8426$. The stimulation effect of metals on the development of the plants in appropriate concentrations has been reported from many authors (9, 22, 52).

The influence of soil extract on the weight of root and stem of the radish *(Raphanus sativus var. radicula)* is presented on **Fig. 6 (b)**. Soil extract didn't affect on the weight of radishes' root, there were no significant difference between control and treated plants. The average weight of root in control plants were 0.911 ± 0.11 g as in the treated with 100% soil extract was 0.963 ± 0.06 g. The stems gain weight under the treatment with the soil extract the linear regression has been received. The correlation index of the linear regression is $R^2 = 0.878$ (**fig. 6**, **b**). The weights of stems in variant-100% were 3.261 ± 0.11 g while in control plants just 2.89 ± 0.11 g with significance $p< 0.05$.



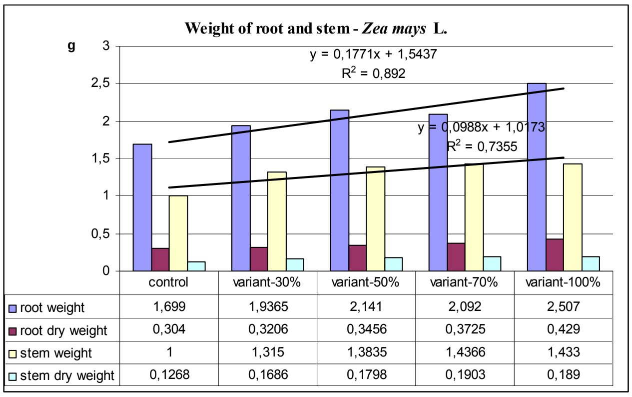
	control	variant-30%	variant -50%	variant - 70%	variant -100%
root weight	0,751	0,62	0,73	1,00	1,06
root dry weight	0,1825	0,16	0,16	0,16	0,14
stem weight	1,34	1,27	1,40	1,60	1,68
stem dry weight	0,16	0,18	0,16	0,15	0,15

a) Influence of the soil extract on the weight of root and stem of wheat sprouts;



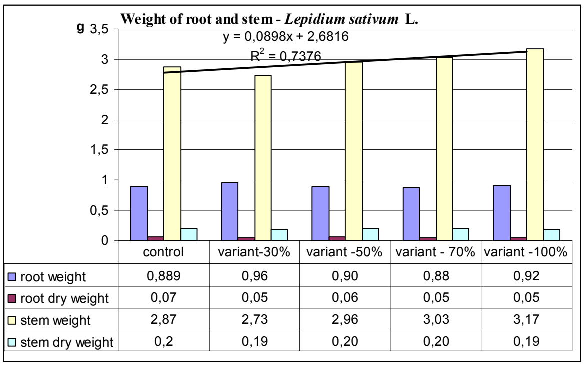
	control	variant-30%	variant-50%	variant-70%	variant-100%
root weight	0,911	1,019	0,9558	0,9378	0,963
root dry weight	0,048	0,054	0,0575	0,13	0,05
stem weight	2,89	2,83	3,04	3,117	3,26
stem dry weight	0,2	0,198	0,2	0,2	0,199

b) Influence of soil extract on the weight of root and stem of radish sprouts;



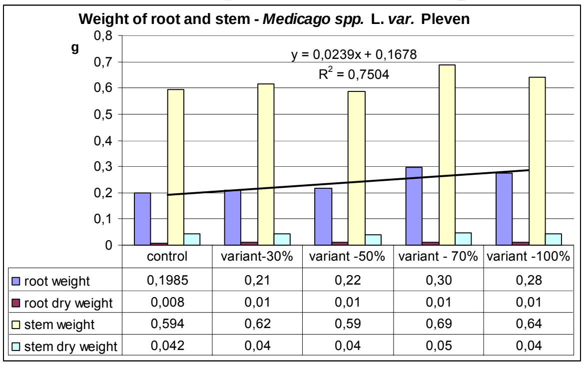
	control	variant-30%	variant-50%	variant-70%	variant-100%
root weight	1,699	1,9365	2,141	2,092	2,507
root dry weight	0,304	0,3206	0,3456	0,3725	0,429
stem weight	1	1,315	1,3835	1,4366	1,433
stem dry weight	0,1268	0,1686	0,1798	0,1903	0,189

c) Influence of the soil extract on the weight of root and stem of maize sprouts;



	control	variant-30%	variant -50%	variant - 70%	variant -100%
root weight	0,889	0,96	0,90	0,88	0,92
root dry weight	0,07	0,05	0,06	0,05	0,05
stem weight	2,87	2,73	2,96	3,03	3,17
stem dry weight	0,2	0,19	0,20	0,20	0,19

d) Influence of the soil extract on the weight of root and stem of *Lepidium sativum* L. sprouts;



	control	variant-30%	variant -50%	variant - 70%	variant -100%
root weight	0,1985	0,21	0,22	0,30	0,28
root dry weight	0,008	0,01	0,01	0,01	0,01
stem weight	0,594	0,62	0,59	0,69	0,64
stem dry weight	0,042	0,04	0,04	0,05	0,04

e) Influence of the soil extract on the weight of root and stem of *Medicago spp*. L. *var*. Pleven;

Fig. 6 Influence of soil extract on the weight of stems and roots

In the conducted bioassay *Zea mays* L showed a pronounce stimulation effect from the influence of soil extract. The plant is tolerant to high metal contend in the soil (52). With increasing concentration of soil extract gradually has been observed increasing the weight of roots, respectively, from 1.69 ± 0.29 g in control to 2.5 ± 0.3 g for variant-100% ($p<0.05$). The linear regression with high correlation coefficient was obtained $R^2=0.892$. The linear regression was attained and for the weight of stems. The correlation index is $R^2=0.7355$, the weight of stems increases from 1 ± 0,2 g in control to 1,4 ± 0,2 g (**fig.6**, **c**).

It has been reported that maize (*Zea mays* L.) can be used as available for phytoextraction and remediation of contaminated with heavy metals soils (52). A plant for this purpose needs be heavy-metal tolerant, grow rapidly with a high biomass yield per hectare, have high metal accumulating ability in the foliar parts, have a profuse root system, and a high bioaccumulation factor. Certain metals (e.g. Cd and Pb) have been reportedly accumulated by the *Zea mays* L above the level used to define metal hyperaccumulation. Maize (*Zea mays* L.) has been evaluated as a widely grown staple cereal with promising attributes of a heavy metal accumulator (52).

The weight of roots between control and treated plants of *Lepidium sativum* L. didn't express significant difference. The stems under treatment gain weight, linear regression with R^2=0.7376 extrapolate the results (**fig.6, d**).

The same trend has been received for the weight of roots and stems of *Medicago spp*. L. *var*. Pleven. There were no significant difference for the weight of stems between treated and control plants. Linear regression with correlation R^2=0.7504 has been observed for the roots of pants.

Generally, in all examined plants stimulus effect under the treatment with soil extract on the weight of roots and stems have been registered.

Conclusions

The negative toxicological effect of wastewater on the soil respiration has been registered. The results of the bioassay showed that all tested concentrations lead to a change in the intensity of breathing of soil ($p<0.05$).

The influence of soil extracts on germination of seeds is associated with reduced jointly germination in treated seeds for all tested plants. Strong negative impact of soil extracts on the energy of germination has been registered for alfalfa (*Medicago* spp. L. *var. Pleven*) seeds. The linear regression with correlation R^2=0,952 has been obtained. The greatest negative impact was observed in 70% extract of soil ($p<0.01$).

Obvious negative effect on the growth of garden cress (*Lepidium sativum* L) roots has been registered for the treatments with wastewater. The obtained results generate a linear regression with correlation coefficient R^2=0.9291. Retardation in the development of roots were 26 up to 62% compared to control, which increases with decreasing the dilution of sewage ($p<0.01$).

In the conducted bioassay *Zea mays* L showed a pronounce stimulation effect from the influence of soil extract. The maize is more tolerant to the metal contamination.

The conclusion is that the sewage from the metallurgical pant "Radomir Metal" is used properly for irrigation of arable land. The question can be which kind of plants is suitable to be cultivated there. The effluent is seems toxic for the aquatic systems and has a slight negative impact on the soil breathing and germination of treated plants. Nevertheless, in the bioassay for all examined plants stimulus effect under the treatment with soil extract on the weight of roots and stems have been registered. The stimulation effect of metals on the development of the plants has been reported from many authors (9, 22, and 52).

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