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POSSIBILITIES OF *SINAPIS ALBA* AS BIO-TESTER AND PHYTO-MEDIATE IN TERMS OF ORGANIC-CHLORINE CONTAMINATION OF SOIL

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Abstract

The results of laboratory microcosmicexperiment to investigate bio-toxic and opportunities of phytore-cultivationof organic-chlorine contaminated soils of toxic waste landfill in Kalush mining area (Western Ukraine) were presented in this article. Research was conducted at the molecular, organic and organismal levels; laboratory speciesof Sinapisalbawas used as a bio-tester. There was proved an inhibitory effect of soil toxicants on growth processes and seed germination of the bio-tester, activation of physiological and biochemical mechanisms of protection. Most informative biomarkers for bio-tests of organic-chlorine contaminationwere defined. The possibility of adjusting the resistance of plants by enriching the soil with bio-universal fertilizers was proven. Sinapisalbawas recommended to be introduced into bio-monitoring system and phyto-remediation of toxic wastedisposallandfill.

Key words: *organic-chlorine pollutants, soil, bio-testing, phyto-remediation,Sinapis alba, Kalush mining area, western Ukraine*

1. INTRODUCTION

Organic-chlorine compounds (OCC), including hexachlorobenzene (C_6Cl_6) and its derivatives are among the most dangerous pollutants of the environment. Hexachlorobenzene (HCB) is a class I of environmental hazardous substances,dioxin-like toxin, which, when it's entered into natural habitats, causes deep, hard to revert violations of structural and functional organization of biological systems at different hierarchical levels. This is because HCB is highly resistant to decomposition, has good migration ability, particularly in trophic chains, has vivid bio-accumulative properties and polytrophic (carcinogenic, teratogen, mutagenic, immune-toxic, etc.) effect on living organisms. Toxicant is a subject to trans-boundarytransfer through all components of the environment by migratory species; can be deposited over large distances from the source of direct entering into ecosystems; it's maximally accumulated in the terminal links of trophic chains; can be harmful to living organisms in very small concentrations. According to the Stockholm Convention dated 2001, HCB belongs to a group of persistent organic pollutants (POP) - a heterogeneous set of artificial chemical compounds with marked toxic properties , the use of which, getting into the environment, distribution and concentration are subject to strict control and regulation (Report on Carcinogens, 2012).

One of the world largest burial places of HCB is in Western Ukraine - Kalush mining areas. Landfill of toxic waste organic-chlorine chemicals by "Oriana- Galev" Ltd. withtotal area of 4.5 hectares operated during 1973 – 2000.As by documented data of 2001it contains 11,352.5 tons of toxic waste, including 11087.6 tons oftars containing about 90% of HCB. Because of non-compliance of hydrogeological and landscape-environmental conditions of the landfill,violation of tightness of card storage, corrosion of metal containers for preserving HCB caused bymoisture, there was a migration of toxins into the soil and groundwater. Despite of active workstoremove HCB and remediation of the landfill, which took place during 2011 - 2013, deep intoxication of soil environment has led to the formation of local geochemical anomaly and excluded the possibility of any form of use of the landfill site and surrounding areas for the long time. Therefore, assessment of technological transformation and finding ways to remediate organic-chlorinecontaminated soils is one of the important ecological problems of Carpathian region. For these reasons, the aim of our study was to investigate the possibility of using *Sinapis alba* as bio-tester andphyto-remediate forsoilsof landfill with HCB in Kalush mining areas.

2. MATHERIALS AND METHODS

There was evaluated bio-toxic and opportunities of phytoremediation of soils at toxic waste landfill in Kalush mining area, which, according to previously conducted chemical and analytical studies , the content of HCB reaches sub-threshold values, or exceeds standard value by 1.5 - 1.7 times (0.03 mg/kg of dry weight (= 30 ng/g)

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by Ukrainian standards). As bio-tester *Sinapis alba*-plants, grown and exposed to soil and soil extracts of the landfill were used. Additionally, bioremediation properties of white mustard were assessed, and possibilities to correct them by adding universal bio-fertilizer to the soil substrate, which was obtained by bioconversion of waste generated by hide-processing companies. The study was conducted under laboratory factorial-static conditions at 20 ± 2°C during 2013 and the first half of 2014.For setting up experiments, terrestrial microcosmic laboratory models were used, produced by the standard method, and microbiological Petri dishes (Fig. 1). For control variants of the experiment, universal soil-mixture was used, made according to the requirements of national standard to evaluate the toxicity of soils (ISO 11269, 2012).

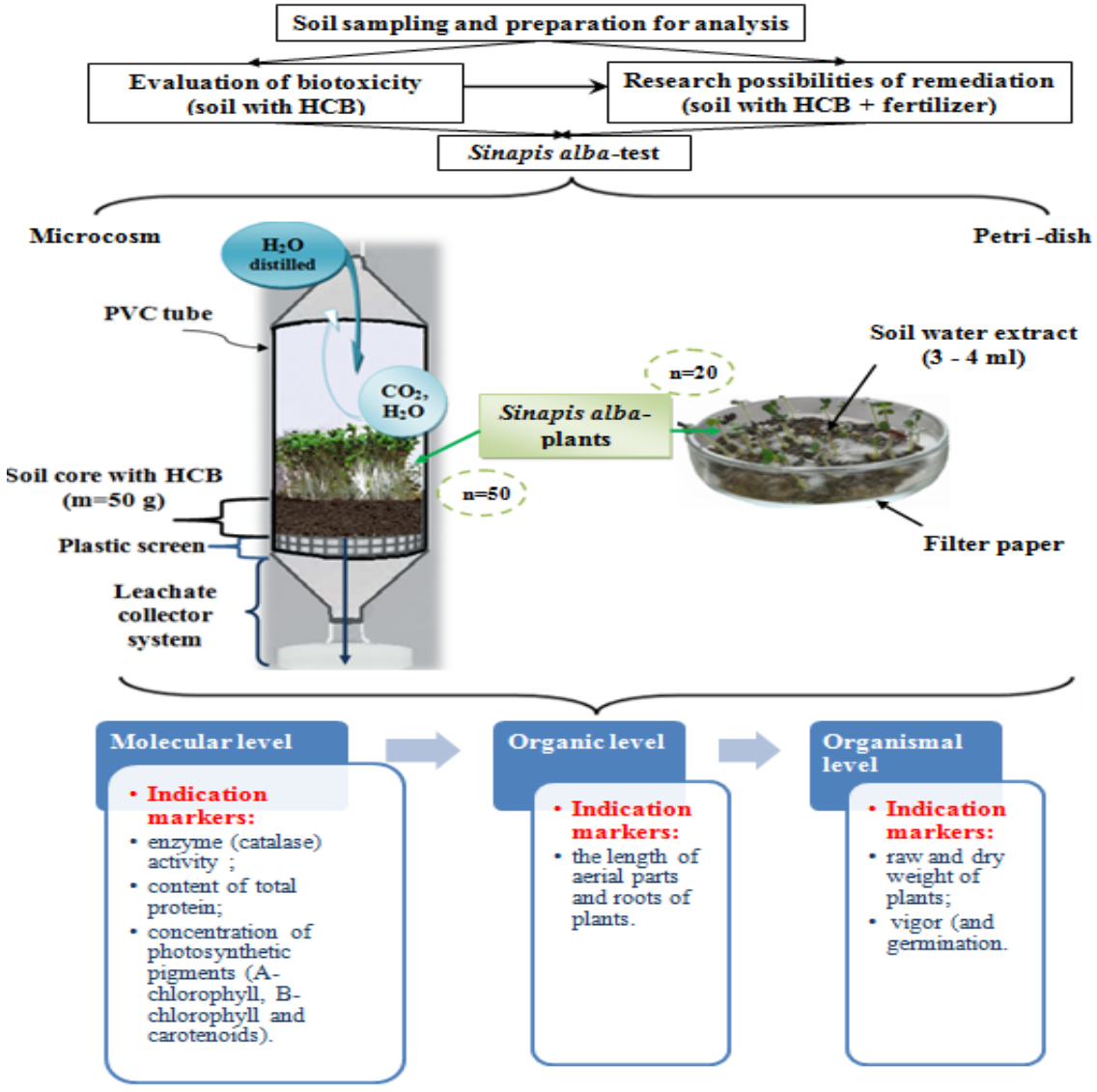


Fig. 1. Conditional diagram of the conducted study to assess soil bio-toxic of the toxic chemical production waste landfill and evaluate the effectiveness of measures using *Sinapis alba* phytoremediation.

Soil sampling was carried out using the "envelope" method in the areas of HCB disposal landfill, which correspond to different stages of the work to remove toxins and reclamation. Geographical location of the landfill and the layout of the sampling locations are shown in Fig. 2. Transportation,handling, storage and preparation of soil for laboratory tests were conducted in accordance with state standards of Ukraine. (ISO 11269, 2012).

To assess bio-toxic of landfill soil and to study the degree of resistance *Sinapis alba* to organic-chlorine contamination, a series of laboratory studies on the molecular, organic and organismal levels were conducted. There were assayed: at the molecular level - enzyme (catalase) activity of anti-per-oxidantsystem of plants and inner concentration of photosynthetic pigments (A-chlorophyll, B-chlorophyll and carotenoids) and content of total protein (10-th day of the experiment); at the organic level - the length of aerial parts and roots of plants, followed by determination of inhibition factor (3-rd and 10-th day of the experiment); on organismal level - raw and dry weight of plants (10-th day), vigor (1st and 3rd day) and germination (7th day of exposure).

Catalase activity was determined by spectrophotometric at a wavelength of 240 nm, recording the change of light absorption by hydrogen peroxide in a sample volume of 2 ml. The mixture, used for reaction contained (final concentrations indicated): KFB - 50 mM (pH 7.0) ,EDTA - 0.5 mM , H2O2 - 10 mM and 5-100 mcl of the supernatant. For calculations molar extinction coefficient for H2O2 - 39.4 M- 1cm -1was used.

(Lichtenthaler, 1997). Specific activity of catalase was calculated formulary,according to the guidelines of state pharmacopoeia (*Государственная фармакопея СССР..., 1990*).

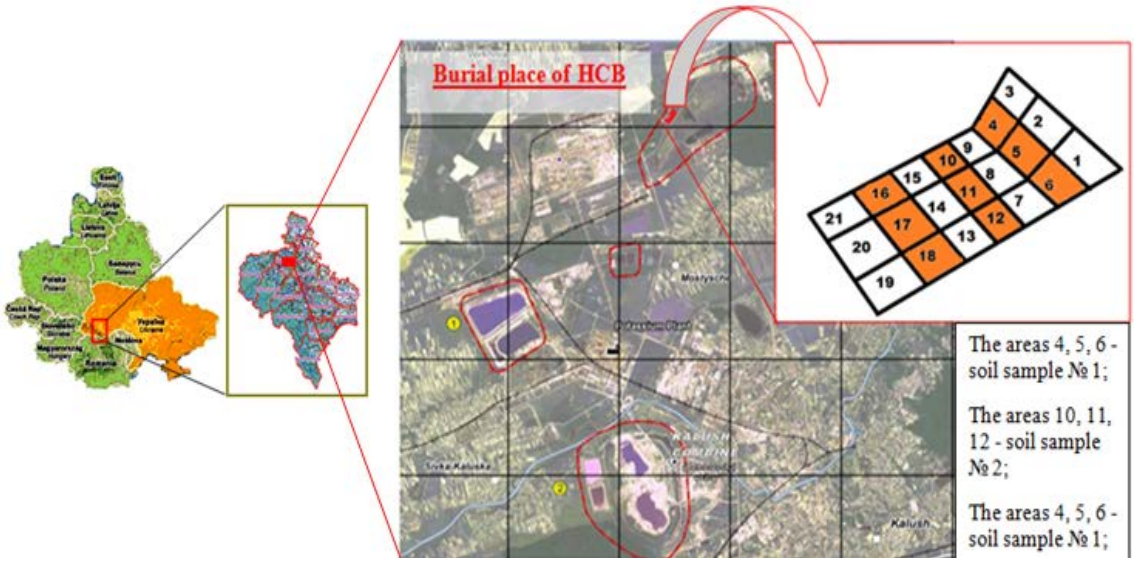


Fig. 2. Geographical location of the toxic waste chemical production landfill in Kalush mining complex and localization of soil sampling locations.

Total protein content was determined using conventional photo-colorimetric method by Bradford (Gitelson, 2001).

Inner concentration of photosynthetic pigments were determined using photo-colorimetric method at wavelengths of 664.2 nm (A-chlorophyll), 648.6 nm (B-chlorophyll a) and 470 nm (carotenoids) using previous extraction with ethanol by centrifugation. The concentration of chlorophylls and carotenoids were calculated using Werner-Vettshteinformulas (Gitelson, 2001).

Linear morphometric parameters of bio-tester (length of the aerial parts and roots) was determined by standard methods with an accuracy up to 1 mm. Inhibition factor (I, %) was calculated as a ratio of variance of morphometric parameters in the control and experimental versions of the experiment to the corresponding control values , expressed as a percentage.

Raw weight of plants was determined by the standard method on an analytical scales with an accuracy of 1 mcg; dry - after preliminary drying of air-dry biomass in thermostatic conditions at a temperature of 100-105 °C for 4-6 hours until the plant reaches its constant weight. To evaluate each biochemical and morphometric parameters 10 plants of each repetition (total - 40 plants per experimental plot) were investigated.

Seeds'vigor was defined as the proportion of sprouted grains to the total number (20 units) in the first and third day of incubation in microcosmic experiment; similarity - similarly, after 7 days of exposure. All studies performed in fourfold repetition with subsequent mathematical and static interpretation and systematization of the results (Терехова, 2011).

Basic statistical values (arithmetic mean value (M), deviation of the arithmetic mean (m)) were calculated by conventional methods. Statistical analysis was complemented by linear correlation one. Statistical significance of differences of the obtained experimental data with background one was evaluated using Student's t-criteriaat various levels of significance α (5, 1 and 0.1 %). The null hypothesis was rejected at $P \leq 0,05$. If the significance level was within $0.10 > P > 0.05$,it was believed that there is a tendency to the manifestation of a process.

3. RESULTS AND DISCUSSION

The results of these studies reveals significant bio-toxic of soils at the HCB disposal landfill inKalush mining areas. Bio-toxic effect is revealed, when testingby*Sinapisalba*,by the change of all analyzed parameters in the pilot versions of the experiment, compared to the corresponding control values, proportional to the concentration of HCB in the soil substrate. Identified bio-tester reactionsare mostlyexpressed at the molecular level and are partially offset when rising levels of bio-system organization.When exhibiting in landfill soils,white mustard plants show up a number of signs of toxic shock and enhancement ofphysiological adaptive mechanisms. One of the elements of bio-tester'sshock response is statistically significant ($0.001 > P < 0.005$) increase in the activity of antioxidant enzyme - catalase. In the control variant of the experiment catalase activity was equal to 32.2 mmolH_2O_2/mh protein/min (Fig. 3).

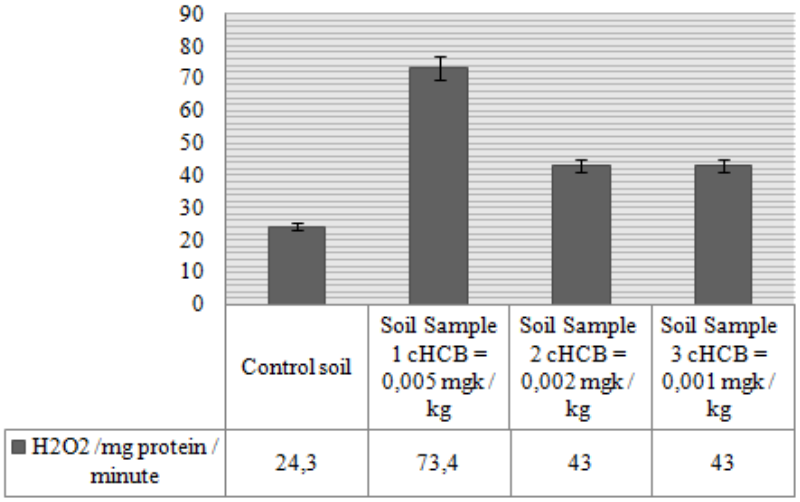


Fig. 3. Catalase activity in *Sinapis alba* plants when exhibited in soils at toxic waste landfill with different concentrations of HCB.

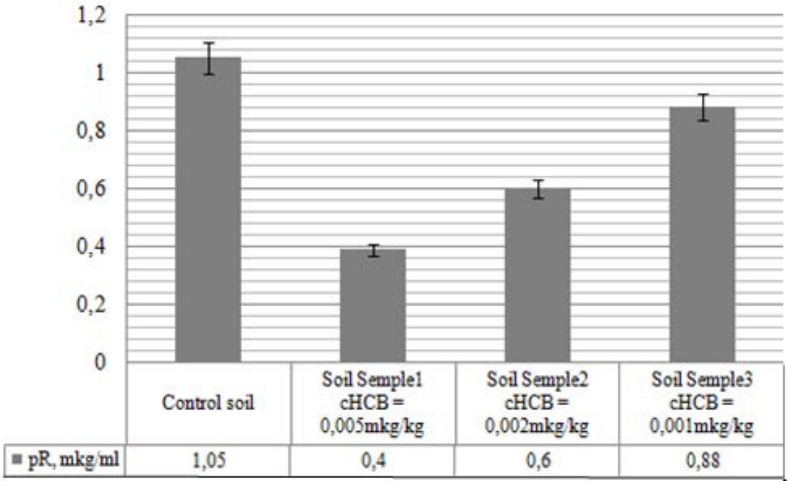


Fig. 4. The total protein content in *Sinapis alba* plants when exhibiting in soils at toxic waste landfill with different concentrations of HCB.

The maximum growth of analyzed indexwas stated under conditions when test object was exposed in soils with the highest content of HCB - 0,005 mg/kg of dry weight (test sample 1). These soil samples are restricted to the area of the landfill within which the decompression of card storage and removal of HCB occurred during the third stage of the work (2013) , that is, shortly before the time of groundwater samples. Presumably, at this point,natural processes of self-healing and detoxification of anthropogenic edafotopwere not occurred yet, signs of which are traced in areas where removal of HCB was carried out in 2010 (soil sample 3) and in 2011 (soil sample 2). For a specified concentration of HCB catalase activity reached 73.4 mmolH_2O_2/mh protein/min, which is evidence of a violation of plant homeostasis and activation of protective functions of bio-tester. When exhibiting *Sinapisalba* in soil samples 2 and 3, the intensity of the protective systems are weaker, however evidence of the impact of stressors are present. Catalase activity of white mustard plants exceeds the reference value by 1.34 times.

Along with increasing antioxidant enzyme activity in leaves of Sinapisalba, there isa decrease of total protein, which is a sign of the prevalence of katabolic processes and is a common feature of physiological stress (Fig. 4).

In the control variant of the experiment,content of total protein is 1.05 mg/ml; in research variant - concentration is significantly lower (P> 0.001) and varies within 0.40 mg / ml in a sample of soil 1 to 0.88 mg/ml in a sample of soil 3.

High reliable values of the coefficients of linear correlation between the content of HCB in soil substrate and catalase activity and total protein concentration (r_{cat} = 0,98; r_{pR} = -0,95), and expressed reaction of analyzed biochemical parameters indicate the prospects of their application in practice of bio-monitor researches and to improve the regulation of organic-chlorine contamination of edafotops.

Photosynthetic pigment complex of Sinapisalbais distinguished by a significant reactionary in terms of organic-chlorine contamination of soil observed (Table1). Changing the spectral characteristics of the pigments-photoreceptors (chlorophylls and carotenoids) is one of the most expressive features of plants adaptation , and therefore can be considered as informative nonspecific marker of environmental stress of habitat in general, and organic-chlorine pollution in particular.

Table 1. Inner concentration of photosynthetic pigments *Sinapis alba* (C, mcg/mg), coefficients of their correlation with the concentration of HCB (r) in the soil substrate

Indication marker / Conditions of the experiment	A-chlorophyll		B-chlorophyll		Carotenoids	
	C,mkg/mg	$R_{chl.}$ [a]	C,mkg/mg	$r_{ch}l$ [b]	C,mkg/mg	r_{carot}.
Control soil	1,552±0,046	-0,95	0,859±0,036	-0,79	0,361±0,013	-0,89
Soil Sample 1	0,656±0,124 α		0,378±0,083 α		0,132±0,003 α	
Soil Sample 2	1,060±0,159 β		0,360±0,065 α		0,280±0,05 β	
Soil Sample 3	1,129±0,135 β		0,625±0,094 γ		0,229±0,06 α	

α - P < 0,001; β - P < 0,01; γ - P < 0,05.

In terms of exposure of test-object in soils at landfill, general trend in change of photosynthetic pigment complex is significant (0.001 > P < 0.005) reduction in the concentration of chlorophyll and carotenoid content, proportional to HCB in the substrate, as compared to the corresponding control values. This reaction is,most likely, related to the ability of toxins to accumulate in chloroplasts, intensifying the processes of free radical oxidation of lipids of their membranes and causing degradation of photosynthetic pigments.

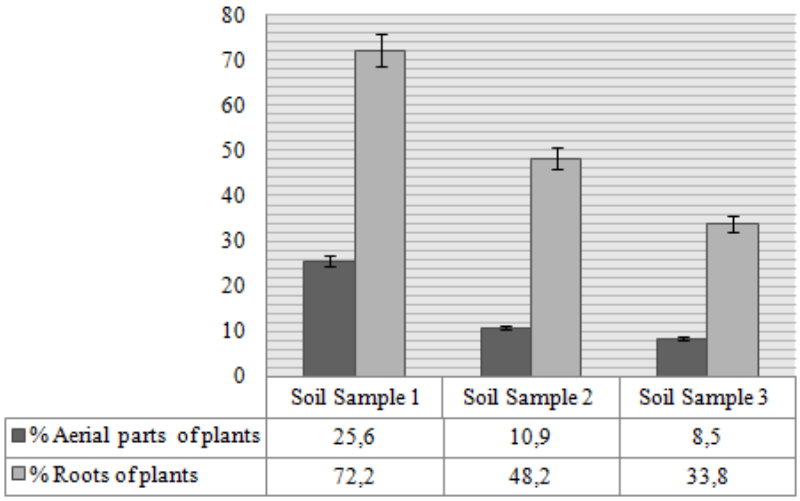
A-chlorophyll is distinguished by maximum variability. The maximum deviation from the control value (1,552 mcg/mg) was ascertained by exhibiting *Sinapis alba* in the soil sample 1 - 0.656 mcg/mg; minimum –in soil sample 3 - 1,129 mcg/mg. Analyzed parameter is distinguished by most close negative correlation with the

concentration of HCB in soil - r_{chl}^{a}= -0.95 . This demonstrates the high indication value of the biochemical parameters for practical bio-monitor researches.

Concentration of B-chlorophyll is more stable indicator. Its concentration fluctuates from 0.378 mcg/mg in sample 1 to 0.625 mcg/mg in sample 2, while reference value of 0,859 mcg/mg. Pearson coefficient of linear correlation between organic-chlorine contamination of soil and inner concentration B-chlorophyll (r_{chl}^{b}) is equal to 0,79.

Carotenoids content in the experimental variants varies in the range of 0.132 - 0.280 mcg/mg withreference value of 0,361 mcg/mg. Correlation analysis reveals the close connection between the negative content of carotenoids in the tissues of the test object and the concentration of HCB in soil r_{carot} = -0.89 . Reduction of the concentration of carotenoids may be regarded as an evidence of depletion of protective features of plant organisms.

At organic level toxic effect of groundwater contaminants becomes apparent by inhibiting of growth processes of bio-tester plant and decreasing morphometric parameters, as indicated by the dynamics of the coefficient –I, %. Inhibiting effect is more pronounced in the root system of *Sinapis alba* (Fig. 5).



	Soil Sample 1	Soil Sample 2	Soil Sample 3
% Aerial parts of plants	25,6	10,9	8,5
% Roots of plants	72,2	48,2	33,8

Fig. 5. Dynamics of inhibiting coefficient of *Sinapis alba* on soil samples of toxic waste disposal landfill.

Inhibiting coefficient increases in direct proportion to the concentration of HCB in the soil and reaches a maximum at bio-tester's exhibiting in soil sample 1 (72.2% - for the root and 25.6 % - for aboveground partof the plant), and a minimum - in soil sample 3 (respectively, 33.8% and 8.53 %). The coefficient of linear correlation between morphometric parameters of *Sinapis alba* and the concentration of HCB in the soil exceeds 0.99. When growing of white mustard in soils of toxic waste landfill, a delay in phenological development stages was also observed, aswell as necrotic lesions of roots and their placement above the soil surface (Fig. 6. A - C).

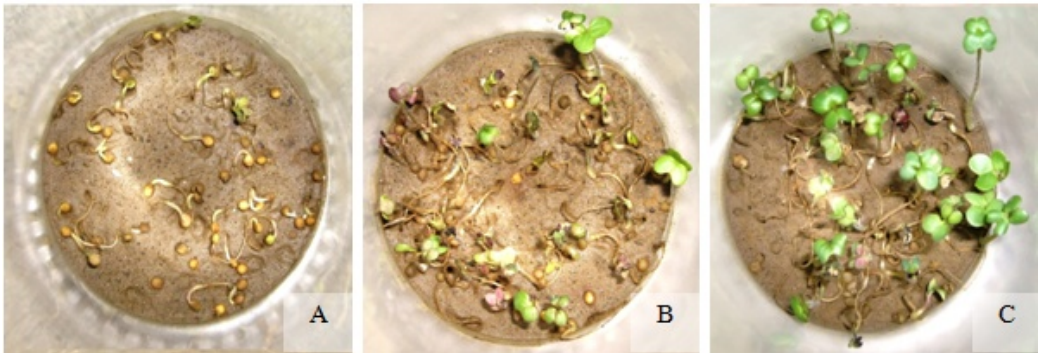
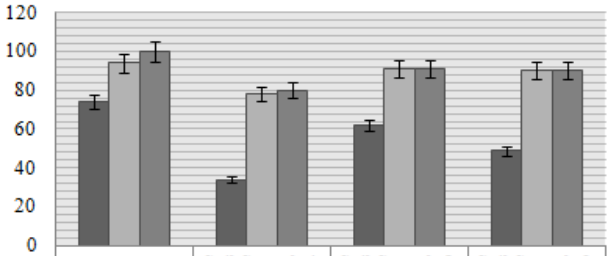


Fig. 6. A - C. Morphological features of *Sinapis alba* in exhibiting at the soil sample site (3th day of exposure): A - Soil Sample 1; B - Soil Sample 2; C- Soil Sample 3.

Along with the decrease of morphometric parameters of bio-tester, a reduction of dry and wet biomass of plantswas also observed. Greater lability was presentin raw biomass, which in the experiment deviated in the range of 0,096 - 0,012 g at the benchmarkof 0,098 g.

At organismal level, we can state certain stabilization of the analyzed model of biological system, as shown by size and nature of changes of the relevant indicative parameters: vigor and germination energy of seeds of *Sinapisalba* (Fig. 7).



	Control soil	Soil Sample 1 cHCB = 0,005 mgk / kg	Soil Sample 2 cHCB = 0,002 mgk / kg	Soil Sample 3 cHCB = 0,001 mgk / kg
Vigor (1st day), %	74	34	62	49
Vigor (3rd day), %	94	78	91	90
Germination (7th day), %	100	80	91	90

Fig. 7. Dynamics of vigor and germination energy of seeds of *Sinapis alba*, exhibited in disposal of HCB soils landfill.

Such a stabilization shows a high efficiency of physiological and biochemical mechanisms of protection, and, in general, indicates the ability of white mustard plants to adapt to organic-chlorine pollution of the environment.

Significant reduction in seeds germination ($P < 0.05$) was set only when exhibiting of bio-testerin soil sample 1 (83.3 % of the control value). In other embodiments, the research noted a tendency to change in the test indicator. Vigor fluctuates over a wide range of values on the first day of incubation (from 45.9 in soil sample 1 to 83.8 in soil sample 3). With increasing of the duration of experiment, the effect is graded. So, on the third day of exhibition, significant difference, relative to control one,was only pronounced in plants germinated in soil sample 1 (83.3 %). The coefficients of Pearsonlinear correlation were negative significantly higher (respectively -0.84 and -0.97).

In parallel, there was explored a perceptiveness of usage of *Sinapis alba* inphytore-cultivation for organic-chlorine contaminated soils and the impact of universal bio-fertilizers on bio-toxic soil environment. The results allow us to conclude significant remediate effect on fertilization of soil contaminated with organic-chlorine compounds. Partial levelingof bio-toxic effectswas observed at all levels of model biological system, as evidenced by the nature and magnitude of change of the applied indicative parameters (Fig. 8).

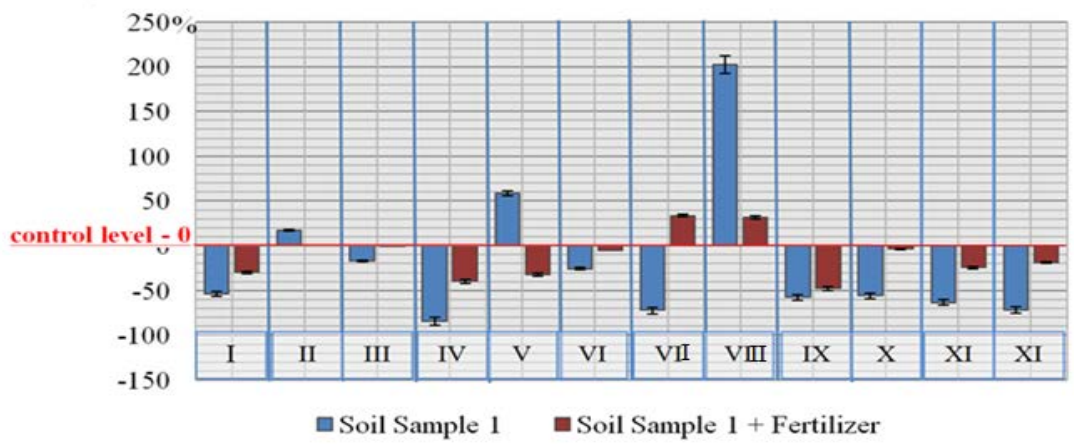


Fig. 8. The difference in numerical values of bioindication markers for *Sinapis alba* exhibited in the soil of disposal of HCB landfill (soil sample 1) without and with addition of bioorganic fertilizer (ratio soil: fertilizer - 1: 1)

I - vigor (1st day); II - vigor (3rd day); III - germination (7th day); IV - raw weight of plants; V - dry weight of plants; VI - length of aerial parts of plants; VII - length of roots of plants; VIII - catalase activity; IX - concentration of B-chlorophyll; X - concentration of B-chlorophyll; XI - concentration of carotenoids; XII - content of total protein.

The positive impact of bioorganic fertilizer is shown at all levels of the bio-system organization. Among the physiological and biochemical bio-indicative markers, remediate effect is maximally reflected by the activity of catalase and the concentration of B-chlorophyll; at organic level - raw biomass and the rate of inhibition of plants bio-testers' roots; at organismal level - seeds' vigor on the first day of incubation. Overall, the addition to the soil of bioorganic fertilizer, obtained by bioconversion of hides' wastes, reduces bio-toxic of organic-chlorine contaminated soils and increases the resistance of white mustard plants to the impact of toxicant.

4. CONCLUSIONS

In soils of the toxic waste landfill at Kalush mining area (Carpathian region, Western Ukraine) content of HCB and its derivatives locally exceeds standard rates, which is a significant environmental hazard to the public and biota. Plot of the landfill and adjacent areas are in need of enhanced environmental control and conduction of scientifically based remedial measures, including phytore-cultivation and bio-remediation.

Soils of the landfill have expressed bio-toxic effect which manifests itself when tested by*Sinapis alba*, via the change of various indicators of molecular (catalase activity, the quantitative composition of the photosynthetic pigment complex of total protein), organic (linear morphometric parameters of aerial parts and roots) and organismal levels (wet and dry biomass, vigor and seed germination). Identified bio-tester's reaction is mostly pronounced at the molecular level and are partially leveled when level of bio-system organization raises. All analyzed parameters are closely correlated with the concentration of HCB in the soil substrate.

All analyzed bio-indicative markers of *Sinapis alba*are observed to be sufficiently informative and can be used in practice of bio-monitor research of organic-chlorine contaminated soils. At the molecular level, the highest reactionary was marked by catalase activity and total protein content; at organic level - morphometric parameters of the roots; on organismal level - vigor on the first day of incubation.

Bringing bioorganic fertilizer to the soil reduces bio-toxic manifestations of organic-chlorine contamination and can be recommended as one of the stages to remediatethe HCBdisposal landfill. Remediate effect is manifested by stabilization of laboratory microcosmic bio-systems and proximity of bio-indication markers to control values.

The relative resistance of *Sinapis alba* to the effects of organic-chlorine pollutants on organic and organismal levels, which is achieved by reactionary of physiological and biochemical adaptation mechanisms, allows us to recommend the species for use in phyto-remediation activities at the toxic waste landfill in Kalush mining areas.

REFERENCES

Терехова, ВА 2011, ' Биотестирование почв: подходы и проблемы' , *Почвоведение*, no 2, pp. 190-198.

Lichtenthaler, HK 1997, ' Chlorophylls and carotenoids: pigments of photosynthetic biomembranes', *Method Enzymol.*, no 148, pp. 331–382.

Gitelson, AA, Merzlyak, MN. &, Chivkunova OB 2001, 'Optical properties and nondestructive estimation of anthocyanin content in plant leaves', *Photochem. Photobiol*, no 74, pp. 38-45.

ISO 11269, Soil Quality – Determination of the effect of pollutants on soil flora – Part1: Method for the measurement of inhibition of root growth, 2th Edition, InternationalOrganization forStandardization, Geneva, 2012

МЗ СССР 1990, *Государственная фармакопея СССР. Общие методы анализа. Лекарственное сырье*, Медицина, Москва, vol. 2, pp. 364 -366.

Mylenka M, 2013, *'Molecular indices of woody plants as bioreagent markers of urboindustrial load (for example, the zone of influence Burshtyn TTP, Ukraine)*, 43rd Annual Meeting of the Ecological Society of Germany, Austria and Switzerland, Potsdam, Germany, P. 201.

Report on Carcinogens, viewed 01 February 2014, < http: //ntp.niehs.nih.gov/ ntp/roc/eleventh /profiles/ s093hexa.pdf>.