INTRODUCTION

Due to the desire to obtain maximal yields, there has been an increase in anthropogenic load on soils, related to the use of physiologically acid fertilizers, pesticides, herbicides, chemical meliorants of different quality degree as well as the use of green manure crops as “substitutes” of manure, which lead to the acidity of soils as a result of fast mineralization of their fresh and highly labile organic matter. At the same time, the soils are prone to stress due to global climate changes, as the expected increase in the soil temperature enhances the dissociation of organic acids in soil, the intensity of the impact of soil biota on the mineralization of organic matter and additional formation of acid-forming oxides of nitrogen, sulphate and carbon. Therefore, at present some of the main diagnostics criteria of the functional

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stability of soils should be its operational efficiency and ability to reflect the “health” of soil [1].

The traditional system of the agrochemical and agro-ecological diagnostics of the state of soils is mostly based on the complex of such agrotechnical and physical-chemical indices as pH level, hydrolytic acidity, the sum of absorbed bases, the degree of saturation with bases, the content of mobile aluminum, the content of heavy metals, biological indices and, less frequently, physical indices. The determination of the content of any substances in soil using traditional analytical methods is a labor- and time-consuming process as it involves selecting and transporting samples and their laboratory and chemical analyses.

We proposed a convenient resource-saving and, more relevantly, rather cheap development – the operational diagnostics of the functional stability of soils under the impact of natural and anthropogenic loads. The diagnostics is made in several stages: potentiometric studies in-situ, simulating the loads (natural and anthropogenic), diagnostics of changes in soil via bioindication using the organisms, sensitive to the environmental changes, and determination of acid-base buffer ability of soils. This method ensures complex estimation of the efficiency of soil functioning [1].

It should be noted that the coefficient of functional stability of soil (K f.st.) as an integral index of physical-chemical (buffer ability and activity of calcium) and biological (activity of protease enzyme) properties of soil was proposed for the first time. This is a substantial factor, taking into consideration the soil as a living biological body which can be presented not via separate components but only via their specific combination [1].

As buffer properties of soils play a relevant role in the stabilization of soil fertility and in the estimation of evolutionary direction of the fertility potential of soils, this property was selected by us as one of the components with the greatest impact on the soil stability. The buffer capacity regarding a specific element of fertility is a reliable criterion of its stability and an actual cumulative index for the estimation of the functioning of the nutrition regime by a specific element of fertility.

The acid-base state of soil is one of key indices of its fertility. The acid-base balance of soils is directly connected to the content of alkaline and alkaline-earth cations as the main antagonists of hydrogen and other elements, which have their impact on the formation of pH level. It is the balance of these elements in soil that defines the vector of direction of soil processes towards acidification or, vice versa, alkalization of the soil medium.

The value of active or potential acidity or alkalinity is often not an objective characteristic of the acid-base function of soils. It is more important in the theoretical and practical sense to have clear determination of the level of susceptibility of soils to the acidification or, vice versa, alkalization. The application of the recent achievements of soil studies towards the use of theoretical and practical provisions of the buffer ability of soils allows eliminating these drawbacks [2].

The example of diagnostics and optimization of the acid-base status of a specific type of soils is presented in the works [3–7].

Talking about the soil stability, it is impossible to omit calcium which is a relevant component of soil stability as a natural body. It is also one of relevant macronutrients of the nutrition of plants, a factor of soil structuring and humus stabilization, and also a regulator of soil acidity. Therefore, the stability of reserves of soil calcium and the optimization of processes of its accumulation-dissipation in soil is an urgent issue. The latter is especially urgent in modern conditions of climatic instability due to the accumulation of carbon dioxide in the atmosphere, and thus – to the hazard of shifts in the carbonate equilibrium.

Soil processes involving calcium are characterized via a group of potentials, among which a prominent place is given to the lime potential pH – 0.5pCa or the potential of hydrogen-calcium exchange.

The methods, based on determining the hydrolytic acidity, saline and water pH in soils, prevail among many current methods of diagnostics and optimization of the acid-base balance in soils.

Soil microorganisms are the pioneers of the soil-forming process. They determine the biological activity of soils, their fertility and ecological status to a large degree. However, at present their ability of serving as indicators of “soil health” is becoming more urgent. It is microorganisms that are some of the first to react to the slightest environmental changes, therefore, their reactions to different pollutants, more often – heavy metals [8, 9], or fluctuations in the existence conditions are very valuable for ecological monitoring, including soil monitoring.

As for microbiota, a considerable role is played by enzymes – organic catalysts of protein nature, accumu-
lated in soil in the process of activity of living organisms. Due to enzymes, there are processes of humus accumulation and restoration in soils, the trophic and sanitary functions are activated, etc. However, natural catalysts are often inhibited due to the following factors: the application of intensified soil tillage [10, 11]; the pollution of soil with pesticides [12]; the formation of specific natural compounds such as tannins [13] and terpenes [14] in soils under forest plants and microcysts in the soils of lacustrine deposits [15]; the pollution of environment [16], etc.

The enzymes take a considerable share of soil fauna, therefore, the improvement of their living conditions is one of the most important tasks of ecological soil studies in current conditions of intensifying agriculture. In particular, the use of the scientifically grounded system of tillage and fertilization of soil [10, 17] promotes the increase in populations of different microorganisms. However, due to popularization of resource-saving methods, there are new ways of optimizing the microbial regime of soils in the world. One of these methods is covering oak (and other kinds) of biochar with the arable layer of soil [18, 19]. It was proven that this leads to the increase in the amount of total organic carbon and microbiological activity, and, as a result, in soil quality.

The degradation processes are of dual nature – natural and anthropogenic. A new issue of soil studies is the recultivation and restoration of disturbed soils of urbanized areas. Recently, there has frequently been a new term in the literature – “urban soils” – anthropogenically modified [20] soils of urban territories, the artificial profile of which has a surface layer of up to 50 cm, created by humans by molding, mixing, burying materials (substrates) of purely urbanogenic origin [21]. Therefore, the functions and properties of such soils are subject to considerable disturbance, especially in terms of functioning of their biological component.

We were interested in the studies on ecologically sensitive soils of urbanized areas (urban soils) with the purpose of activating their biological component and, as a result, partial self-restoration. The cultivation of several kinds of energy crops was suggested as they have a positive influence on the status of soil fauna and the activity of protease enzyme [22, 23] not only on urban soils but also on a number of other types of soils. It is noteworthy that such energy crops as giant miscanthus (Miscanthus Giganteus) and willow (Salix) are also decorative plants, which is especially valuable while cultivating them in urbanized zones.

Protease activity is one of integral indices of the total biological activity of soils, a potential ability of soils to decompose proteins and peptides [24]. Protease takes part in the mobilization and circulation of nitrogen. The higher the content of mobile nitrogen and other elements of nutrition in soil is, the more active the process of cellulose oxidation is. The cellulose-decomposing microorganisms ferment the fiber, synthesize and partially release aminoacids into the medium.

Protease is considered to be one of the most important enzymes in the soils of chernozem type, but one should not underestimate its positive action in other soil types as well. The determination of protease activity in acid and forest soils, the soils which suffered from degradation processes is an efficient method of soil-ecological monitoring. The degraded soils present a wide field for studies in the sphere of ecological soil studies, as they are the first to be subject to recultivation, which are ecologically safe due to their susceptibility [25].

The biological restoration and maintaining the fertility of soils, both degraded ones and those with undisturbed structure, is impossible without the consideration of microbiological, and thus enzymatic component. The special place in our work is given to protease as an enzyme, taking part in the transformation of nitrogenous compounds. The determination of protease activity was paid much attention in the previous century, but even after many years of studies there are many gaps in the literature regarding the response of protease to foreign factors [26]. Current methods of estimating the activity of this enzyme are characterized by much labor and the application of rather a large amount of reagents and devices. In addition, the deviations of these analyses are rather substantial.

We have improved Mishustin’s method of determining protease activity and elaborated an accurate express-mechanism of determining a quantitative index of protease activity of soil, which ensures considerable facilitation and acceleration of current methods and obtaining objective data about the soil quality. It is also important that this method is easily applied in practice with just a required minimum of materials. Therefore, the suggested method has considerable advantages compared to current ones.

It would be reasonable to determine the advantages of the methods, suggested by us, with current ways of determining the abovementioned indices.

First of all, the determination of any soil indices directly in the field is surely a faster and less expensive
method, because it does not require time and material resources for the preparation and transportation of samples, the acquisition of reagents, etc. Therefore, the advantage of the potentiometric method of studying the acid-base status of soils is obvious.

Up till now there have been several methods of determining the biological activity of soils, but all of them are rather complicated in terms of implementing, and the process of obtaining results is very time-consuming. The following methods have proven to be most commonly used: the method, based on the application of cellulose standards [27]; the method using the intensity of flax linen decomposition [28]; the method of determining the proteolytic activity of soils using photopaper or photofilm [29].

Taking into consideration the fact that in modern world the preference is given not only to quality but also to the rate of obtaining the results, the following method of estimating changes in the ecological status of soils will ensure considerable acceleration of the ecological monitoring of soils of different genesis and, as a result, the restoration of their physical and biological properties.

MATERIALS AND METHODS

Potentiometric, biological methods and the methods of determining acid-base buffer capacity were used during the studies.

The estimation of acid-base status of soils is done efficiently via direct potentiometric determination using ion-selective electrodes, as it allows estimating directly in soil without taking a sample, and it does not require any processing of soil material in the laboratory conditions except for diluting with water.

The activity of a substance in soil, determined potentiometrically, is active content of this substance in the aqueous phase in the field soil, or in conditions, maximally close to the field soil.

The activity is usually measured in milligram-equivalents per one liter of soil solution: $a$, [mg-eq/l], or presented in the form of a logarithmic index, similar to pH: $pa = - \log a$. To transfer to milligrams per liter, one should multiply the value of $a$ by atomic or molecular mass of the investigated element, referred to the unit of its valency (equivalent mass). To transfer to the content in soil, one should consider the content of aqueous phase in it (humidity) [2].

As for exchange cations of calcium ($\text{Ca}^{++}$), the activity should be interpreted as active content of mobile water-soluble form.

Any potentiometric estimation is efficient for direct study of the dynamics in the mobile substance in soil as well as for the determination of the spatial variability (diversity) of this content – for instance, to find “spots” in order to correct the norms of fertilizers in the fields which is especially relevant in the practice of directed agriculture [2].

At present, there is a common method of potentiometric determination of pH. Calcium may be determined according to DSTU 4725:2008.

The method of determining the acid-base buffer of soil is based on estimating the change in pH of the soil suspension due to the addition of increasing doses of acid and alkali. It is determined according to DSTU 4456:2005. The results are presented in a graphic form as a dependence of the dose of additive (ad). The obtained curve of pH-buffer capacity is the basis for normative forecast of the needs of chemical melioration of some soils.

The acid part of the buffer capacity for acid soils was taken by us as negative part of pH-buffer capacity and the alkali part – as a positive part. The main indices of pH-buffer capacity of soils are as follows:

- buffer capacity of soils in the alkali part (interval) of loads ($BC_{AI}$);
- buffer capacity of soils in the acid part (interval) of loads ($BC_{AC}$);
- the coefficient of buffer asymmetry ($CBA$) – the ratio of the difference and sum of the above-mentioned capacities

$$BC_{AI} - BC_{AC} \quad BC_{AI} + BC_{AC}$$

- the total estimation index of buffer capacity (TEIBC), which includes the sum of buffer capacities with the consideration of the asymmetry coefficient, determined by the formula:

$$BC_{AI} + BC_{AC}(1-|CBA|)$$

The lower the asymmetry coefficient is, the higher is the rate of the reverse processes or the rate of self-regulation of genetically inherent acid-base balance of soils.

The diagnostics and optimization of the acid-base status of specific soil was conducted in the following way:

- determining optimal values of pH of the soil solution for crops;
IMPROVEMENT OF METHODS OF ESTIMATING THE CHANGE IN THE ECOLOGICAL STATE OF SOILS

– building the chart of pH-buffer capacity for specific soil, distinguishing the optimal pH zones on the chart;
– determining the optimal pH level of soil within a specific crop rotation, reaching which requires the estimation of the meliorant dose;
– calculating and introducing the corresponding dose of the meliorant into soil to reach the given rate.

The estimation of the enzymatic activity by the indices of protease activity was performed by the modified photoautography method on the basis of the laboratory of fertility of hydromorphic and acid soils of NSC IS-SAR named after O. N. Sokolovsky. The abovementioned technology is based on Mishustin’s method of determining protease activity [28].

Photofilms were used as application material. We have improved the method of estimating the protease activity using the graphic editor Adobe Photoshop.

The estimation of proteolytic activity requires the following items: soil samples (50 g – the mass of one sample); Petri dishes; unexposed film; distilled water.

A piece of film is placed on the bottom of a Petri dish (films of 2.5 cm × 7.5 cm were used in our study) with the gelatin layer upwards. Maximally homogenized soil with the weight of 50 g is placed onto the film. One of our tasks was to determine the rate of complete decomposition of the gelatin layer of the film, therefore, we moistened soil samples with distilled water up to 80% from the complete water capacity of soil.

The films were extracted 3–5 days later, depending on the type of soil. For further estimation of the quantitative value of protease activity, the films are carefully washed with distilled water, dried and fixed to carton, preferably of black or yellow color (the most convenient colors for further work in the graphic editor). The obtained films are scanned. The film with non-decomposed gelatin is taken as the control.

The scanned image is opened using the graphic editor Adobe Photoshop. A graphic document is created with the sizes of 2.5 × 7.5 cm with transparent background and the resolution of 78.74 pixels per one centimeter (px/cm). The area with the photofilm is outlined on the scanned image, copied and pasted into a previously created file, then scaled.

Using the instrument “Select → Color range”, select the color of the background (which is the decomposed layer of gelatin) with an eyedropper, here the value of the command “the range” should be 200 for maximal accuracy. Then, the instrument “Histogram” should be opened, which indicates in pixels the areas of the whole image (in our case this is 116427 pixels), and of the decomposed gelatin layer – i.e. the immediate background of the image. Using the ratio of the areas of variants with the decomposed gelatin and the control sample, we receive the value of the total biological activity of soil in percentage.

The final stage of operational diagnostics of the functional activity of soils is the actual calculation of the coefficient of their functional stability. Due to the fact that the acid-base buffer capacity of soil is an integral index, reflecting the changes therein (fluctuations in pH and pCa) and soil capacity to counteract the external load, this index should be one of the main ones to determine the soil stability.

However, it is impossible to have objective estimation of the soil reaction to the exogenous impact, neglecting the living component of soil. Therefore, we proposed the following variants of the formula of calculating the coefficient of functional stability of soils (K f.st.), based on their genetic features:

– for turf-podzolic soils – K f.st. = 0.4 × B + 0.6 × P;
– for gray forest soils – K f.st. = 0.6 × B + 0.4 × P;
– for podzolic chernozem – K f.st. = 0.7 × B + 0.3 × P;
– for meadow soils – K f.st. = 0.5 × B + +0.5 × P;

where B – the ratio of the value of the total estimation index of buffer capacity (TEIBC) of the investigated soil under the impact of anthropogenic or natural loads and the value of TEIBC of the same soil without any loads; P – the ratio of the value of protease activity of the investigated soil under the impact of the anthropogenic or natural loads and the value of protease activity of the same soil without any loads.

Taking into consideration the fact that turf-podzolic soils have the smallest buffer capacity, its role in maintaining the functional stability of soils will be less significant compared to gray forest and chernozem soils. Meadow soils are distinguished by high buffer capacity and considerable biological diversity, i.e. these indices have equal impact on their properties. Therefore, we suggested the abovementioned ratio of the index of the buffer capacity – TEIBC and the biological index PA, the combination of which is the coefficient of the functional stability of soils.
RESULTS AND DISCUSSION

It is noteworthy that the results of further estimations will depend on the quality of scanned image and the color of the background. Further visualization requires editing the image in the graphic editor, as shown in Figure 1.

Below is the algorithm of calculating the proteolytic activity for images with the resolution of 78.74 px/cm and the films with the size of 2.5 × 7.5 cm:

the area of one square centimeter in pixels: \( S = 78.74 \times 78.74 = 6200 \) px; the area of the film in pixels: \( S_{\text{film}} = 116427 \) px.

Checking: a) \( S_{\text{film}} = 2.5 \times 7.5 = 18.8 \) sq.cm; b) \( S_{\text{film}} = 116427/6200 = 18.8 \) sq.cm;

\[ \text{proteolytic activity in } \%: \text{PA} = \frac{S_g}{S_{\text{film}}} \times 100. \]

Based on the data obtained, the analysis of different kinds of soils demonstrated that it is possible to compare the results of the control and the obtained variants of the experiment in laboratory conditions with the indicated moisture capacity as early as 3–4 days later. Table 1 presents the data of observations of the process of decomposition of the gelatin layer of the film in soils on the third day of the experiment. According to the results obtained, it is possible to check the sufficiency of the process duration of 3 days as on the 4th–5th day the gelatin layer is almost completely destroyed on all the films.

In addition, this method may be used to determine the rate of protease action in different soils under various tillage conditions and under different loads, as shown in the charts below (Fig. 2–3).

Table 2 demonstrates the change in the coefficient of functional stability of soils under the impact of anthropogenic loads on the soils of agricultural purpose, and the change in the coefficient on the soils (including degraded ones) where crops are not cultivated. In addition, we calculated the coefficient of functional stability of soils while cultivating energy crops.

Table 2 demonstrates that the highest stability is notable for chernozem. Turf-podzolic soils are more inclined to losing functional stability under the impact of external loads.

CONCLUSIONS

Current methods of estimating the protease activity of soils were optimized which allowed reproducing this

| Name of soil                        | PA I, % | PA II, % | PA III, % | Mean, % | HCP \(_{05}\), % |
|------------------------------------|---------|----------|-----------|---------|-----------------|
| Typical moderately washed chernozem| 76.90   | 73.59    | 75.72     | 75.40   | 1.32            |
| Common moderately washed chernozem | 51.54   | 49.03    | 50.53     | 50.37   | 1.87            |
| Turf-podzolic                      | 4.85    | 5.62     | 5.15      | 5.21    | 2.02            |
| Meadow alluvial                    | 45.05   | 45.18    | 44.65     | 44.69   | 4.04            |
| Meadow-swamp alluvial              | 34.55   | 37.12    | 34.13     | 35.27   | 5.15            |
| Chernozem urban soil               | 39.30   | 39.81    | 38.18     | 39.10   | 2.11            |
| Lithozem urban soil                | 38.44   | 41.91    | 40.74     | 40.36   | 2.81            |
| Chernozem-meadow urban soil        | 70.67   | 72.07    | 71.36     | 71.37   | 4.58            |
analysis using a personal computer and a few laboratory devices.

In modern conditions of intense increase in the anthropogenic load on environment, some of the main criteria of diagnostics of the functional stability of soils should be its operational ability, as many types of soils are susceptible to changes due to external loads, thus, there is an urgent need for timely revelation of these changes with the purpose of efficient management of their fertility.

The algorithm of estimating the functional stability of soils was suggested for the first time. It includes a number of simple stages which do not require much labor or cost and allow obtaining the data regarding specific type of soil in a short period of time. The stages are as follows:

diagnostics of acid-base status of soils using ion-selective methods and determining the rates of activity of calcium ion and pH of soil;
simulating the loads (natural and anthropogenic ones) on soils;
diagnostics of changes in the biological activity of soils using the operational method of determining the protease activity in it;
determining the changes in the acid-base buffer capacity of soil under the impact of loads;
calculating the coefficient of functional stability of soils.
Table 2. The functional stability of soils with different acid-base balance under the impact of anthropogenic loads

| Variant            | TEIBC, points | PA, % | K f.st. |
|--------------------|---------------|-------|---------|
| **Turf-podzolic soils** |               |       |         |
| Control            | 13.3          | 37.0  | 1.00    |
| NPK                | 11.4          | 25.0  | 0.74    |
| Wet lime           | 15.0          | 36.0  | 1.03    |
| Green manure crops | 11.8          | 20.0  | 0.67    |
| Energy crops       | 12.1          | 39.0  | 0.99    |
| **Gray forest soils** |             |       |         |
| Control            | 16.4          | 42.0  | 1.00    |
| NPK                | 14.2          | 36.0  | 0.86    |
| Wet lime           | 18.0          | 30.0  | 0.94    |
| Green manure crops | 16.0          | 38.0  | 0.95    |
| Energy crops       | 16.2          | 40.0  | 0.98    |
| **Chernozem soils** |           |       |         |
| Control            | 30.2          | 50.0  | 1.00    |
| NPK                | 28.2          | 45.0  | 0.92    |
| Wet lime           | 29.0          | 39.0  | 0.91    |
| Green manure crops | 30.0          | 58.0  | 1.04    |
| Energy crops       | 30.1          | 63.0  | 1.10    |
| **Meadow soils**   |               |       |         |
| Control            | 38.7          | 63.0  | 1.00    |
| NPK                | –             | –     | –       |
| Wet lime           | –             | –     | –       |
| Green manure crops | 36.4          | 61.0  | 0.95    |
| Energy crops       | 38.0          | 65.0  | 1.01    |

The proposed operational diagnostics ensures complex demonstration of the efficiency of functioning of the soil, and thus fast elaboration of management events to improve the indices of soil with the purpose of receiving high yields of crops.

Due to the abovementioned method, we calculated the coefficients of functional stability of soils of different genesis. The results obtained confirm high stability of chernozem and meadow soils. The abovementioned K f.st. for turf-podzolic and other sensitive soils under different loads may serve as an instrument of improving their properties.

Удосконалення методів діагностики зміни
екологічного стану ґрунту
під впливом зовнішніх навантажень

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Мета. Оптимізація та прощення вже існуючих методів екологічної діагностики ґрунтів різної генези під впливом різноманітних зовнішніх навантажень. Методи. Лабораторно-модельні (фізико-хімічні, біологічні): визначення кислотно-основної буферності ґрунту (ДСТУ 4456:2005); визначення активності іонів кальцію (ДСТУ 4725:2008); визначення активності протеази за модифікованим методом Мішустіна. Результати. Запропоновані нами методи знижують екологічні діагностування ґрунтів, незважаючи на їх походження та зовнішні чинники. Завдяки цьому спрацьовує моніторинг ґрунтових ресурсів та виявляються фактори, що негативно, чи позитивно, впливають на перебіг ґрунтових процесів. Дані методи покладені в основу «Методології операцівної діагностики впливу природних та антропогенних навантажень на функціональну стійкість ґрунтів» та можуть бути
ИМПРОВЕНИЕ МЕТОДОВ ОЦЕНКИ СТРУКТУРНОЙ ИЗМЕНЕНИЯ ЭКОЛОГИЧЕСКОГО СОСТОЯНИЯ ПОЧВ

ОЦЕНКА ИНДИВИДУАЛЬНОЙ ПРОТИВОДЕЙСТВИЯ И СОБСТВЕННОЙ СПОСОБНОСТИ КАЛЬЦИЙНОЙ СФЕРЫ СОЕДИНЕННОЙ С ПОЧВЕННЫМ ПОДЛОЖКОЙ У ПОЧВЕННЫХ СОЕДИНЕНИЙ

Ключевые слова: функциональная стабильность, буферная здатность, активность протеаз, коэффициент функциональной стабильности.

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Ключевые слова: функциональная устойчивость, буферность, активность протеаз, коэффициент функциональной устойчивости.

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