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# UREASE ACTIVITY OF EDAPHOTOPES ON ROCK DUMPS OF COAL MINES OF THE DONETSK PEOPLE'S REPUBLIC

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## ABSTRACT

The aim of this work is to study the influence of phytorecultivation measures on changes of urease activity of edaphotopes at coal mine dumps of the Donetsk People's Republic. To study urease activity, monitoring sites were selected in the territories of coal mine dumps of varying degrees of degradation. To assess the influence of monospecies herbaceous phytocenoses on the course of soil-forming processes in edaphotopes of anthropogenically transformed ecosystems, seeds of *Kitaibelia vitifolia* Willd., *Onobrychis arenaria* (Kit.) DC. were sown. The research was carried out throughout 2021. Analysis of the data obtained showed that urease is confined to underlying genetic horizons, which is explained by the shortening of the soil profile and the leaching of soluble ammonium salts from the upper horizons. The study of the seasonal dynamics of the intensity of enzyme functioning showed a parabolic nature of changes in enzymatic activity with a minimum in the summer period of research and a maximum in the spring, during the period of enrichment of the soil with organic residues of the previous growing season. Sowing plants in monitoring areas had a positive effect on the processes of initial soil formation. Thus, when using *Onobrychis arenaria*, urease activity increased on average by 18-104% due to the fixation of atmospheric nitrogen by legumes, while in the variants of experiments using *Kitaibelia vitifolia* its activity increased by only 9-64%.

**Keywords**: *dumps, enzymes, phytorecultivation, soil, technogenous ecosystems, urease.*

## INTRODUCTION

The acceleration of the pace of technogenesis and a significant increase in the number of anthropogenic objects polluting the environment certainly has a negative impact on natural landscapes. Extraction and processing of rock mass and rocks through mining leads to the alienation of significant areas for rock dumps, which, in turn, require recultivation. One of the effective and inexpensive methods of eliminating the harmful effects of man-made objects, in particular coal mine dumps, on the environment is phytorecultivation. Currently, there is a process of intensive improvement of recultivation methods not only from the point of view of environmental protection, but also economic efficiency (Syschikov et al., 2020). Soils of technogenic ecotopes belong to a special category of soils, which only in a number of characteristics can resemble real soils. Phytorecultivation is a mechanism that makes it possible to bring the soil conditions of technogenic ecotopes closer to the conditions of natural landscapes (Agurova and Syschikov, 2021).

When studying the influence of monospecific communities of *Kitaibelia vitifolia* Willd. and *Onobrychis arenaria* (Kit.) DC. pH indicators of edaphotopes of coal mine dumps show the prospects of using phytomeliorants in the conditions of technogenic ecotopes to change the reaction of the environment (toward slightly acidic or neutral), which in turn will improve the availability of mineral nutrition elements and help expand the list of plant species used for reclamation (Agurova and Syschikov, 2021). In addition, when carrying out a number of phytorecultivation works, it was established that the prevailing trend is an increase in the concentration of mineral nitrogen compounds in the soil (Syschikov at al., 2021), a positive effect was established with the influence of plants of the Poaceae on the content of organic matter (Syschikov and Agurova, 2020).

The enzymatic activity of soil is one of the main indicators of its fertility. Any soil is characterised by a certain level of enzymatic activity, determined by the diversity and quantitative content of enzymes (Shvakova, 2013). Soils that are constantly experiencing significant anthropogenic impact are characterised by a changed qualitative and quantitative composition of microorganisms; tend to reduce biological activity, which leads to the transformation of biogeochemical cycles of nutrients A number of authors have shown that enzymatic activity reflects the direction and intensity of the processes of biochemical transformations occurring in the soil (Holik et al., 2019; Utobo, Tewari, 2015; Wang et al., 2015).

The activity of soil enzymes is used as a diagnostic criterion of soil fertility, and changes in enzyme activity indicate anthropogenic impact. Enzymes demonstrate high stability during long-term storage; this fact, as well as the low experimental error, contribute to the use of enzymatic activity parameters as a diagnostic indicator of soil condition (Shorec and Balaeva-Tihomirova, 2018).

A number of scientific works, including dissertations, are devoted to the study of the enzymatic activity of soils. Thus, at the Faculty of Forestry and Wood Technology at Mendel University in Brno, Czech Republic, research is being carried out to study the enzymatic activity of soil after long-term use of inorganic and organic fertilizers (Holik et al., 2019). The participation of soil enzymes in the transformation of organic compounds into forms that are easily absorbed by plants, as well as their participation in metabolic processes that occur in the soil, was studied by American scientists (Martinez et al., 2021). The effect of soil fraction size on the activity of soil enzymes was investigated. The Faculty of Natural Resources and Soil Sciences at the University of Gondar, Ethiopia, has also addressed the issue of restoring degraded soils (Fentie et al., 2020).

Urease is an enzyme that catalyses the hydrolysis of urea into $CO_2$ and $NH_3$ with a reaction mechanism based on the formation of carbamate as an intermediate. The

ammonium cation $NH_4^+$ formed as a result of the urease reaction is a direct source of nitrogen nutrition for plants, therefore urease activity is one of the most important indicators of biological activity and soil quality (Povolockaja, 2020; Utobo, Newari, 2015).
The aim of this work is to study the influence of phytorecultivation measures on changes of urease activity of edaphotopes at coal mine dumps of the Donetsk People's Republic.

## MATERIAL AND METHODS

To study urease activity, monitoring sites were selected in the territories of coal mine dumps of varying degrees of degradation. When choosing them, factors such as the prevalence of the type of disturbance within the study site, the degree of anthropogenic transformation, the possibility of restoring biological productivity and involvement in economic activity, and the potential environmental effect when carrying out reclamation activities were taken into account. To assess the influence of monospecies herbaceous phytocenoses on the course of soil-forming processes in edaphotopes of anthropogenically transformed ecosystems, seeds of *Kitaibelia vitifolia* Willd., *Onobrychis arenaria* (Kit.) DC. were sown on an area of 1 $m^2$ separately at each of the monitoring sites.
Based on the results of the studies, it has been shown that the soils of rock dump ecosystems are characterized by a strongly acidic or acidic reaction of the environment (pH varies depending on the horizon and site in the range from 4.06 to 4.92), which complicates the process of colonizing the dump with plants. According to this indicator, the worst conditions develop on the slopes of the dumps, while in the zone of flattening of the dump slope the reaction of the environment is slightly alkaline (pH 7.02) (site No 2).
In terms of organic matter content, we classify the zonal soil as moderately humified (3.38%), all soils in rock dump ecosystems are characterized by a low humus content, not exceeding 0.45%, and therefore we classify it as very weakly humified. The humus-accumulative horizon of zonal soil (site No 1) is characterized by a high supply of mobile phosphates (18.45 mg/100 g of soil). Ecosystems of coal mine dumps are characterized by very low and low contents of available phosphorus (the amount varies from 0.63 to 3.63 mg/100 g of soil). The humus-accumulative horizon of the control plot is characterized by an increased content of exchangeable ammonium (5.75 mg/100 g of soil). For rock dump ecosystems, the supply of ammonium nitrogen is very low (the range of variation in its amount is from 0.97 to 2.61 mg/100 g of soil). Only the humus-accumulative horizon of site №1 is characterized by a high content of nitrate nitrogen (3.3 mg/100 g of soil). For rock dump ecosystems, the supply of nitrate nitrogen is very low—the content does not exceed 0.5 mg/100 g of soil.
The research was carried out throughout 2021 (spring, summer, autumn). Soil samples were selected according to genetic horizons (Zvjagincev, 1991) from localities with natural vegetation cover and in places of experimental sowing of

phytorecultivators a year after sowing. The description of soil sections was carried out according to generally accepted methods (Rozanov, 1983).

**Monitoring site No 1.** The adjoined territory to the southern part of the dump of mine № 12 "Naklonnaya" (Proletarsky district, Donetsk). Total projective coverage 95-100%. *Elytrigia repens* (L.) Desv. and *Vicia cracca* L. growing in groups are dominated. *Artemisia absinthium* L., *Verbascum lychnitis* L., *Achillea pannonica* Scheele, *Euphorbia virgata* Waldst & Kit. are found scatteredly. *Linaria vulgaris* L. and *Pilosella echioides* (Lumn.) F. Schult & Sch. Bip are grown singly. Of the ephemerals, the following species are noted: quite a lot of *Holosteum umbellatum* L. and scattered *Lepidium perfoliatum* L.

**Section No 1.** Medium-humused ordinary chernozem usual.

$A_1$, 0-47 cm – fresh, dark brown, homogeneous, light loamy, medium-grained, moderately thick. No inclusions are noted. There are lots of roots. The transition to horizon $A_2$ is clear in color and structure.

$A_2$, 47-86 cm – fresh, light chestnut brown, heterogeneous, light loamy, medium-grained, moderately dense. No inclusions are noted. The roots are rare. The transition to horizon B is abrupt in color and structure.

B, 86-110 cm – dryish, light maroon color, homogeneous, loamy, lamellar, moderately thick. No inclusions are noted. Sporadic roots. Transition to horizon C tongue-like in color.

C, deeper than 110 cm – dryish, brownish-orange, homogeneous, loamy, lamellar, thick. No inclusions are noted. There are no roots.

We consider this site as a nominal control.

**Monitoring site No 2.** Zone of flattening of the slope of southern exposure at the base of the coal rock dump of the Lenin mine (Gornyatsky district, Makeevka). The phytocenosis is represented by *Echium vulgare* L., *Sideritis montana* L., *Stachys transsilvanica* Schur, *Anisantha tectorum* (L.) Nevski, *Calamagrostis epigeios* (L.) Roth, *Poa compressa* L., *P. bulbosa L., Galium humifusum* M. Bieb., *Daucus carota* L., *Achillea pannonica, Artemisia absinthium, A. austriaca Jacq.*, *Centaurea diffusa* Lam., *Senecio vernalis* Waldst. & Kit.,, *Linaria maeotica* Klokov, *Phragmites australis* (Cav.) Trin. ex Steud., the total projective cover is 50-60%, there are clearings that are not overgrown, the dominance of individual species is not expressed, among woody plants, in addition to the recultivator *Robinia pseudoacacia* L., self-seeding *Fraxinus pennsylvanica* Marsh, *Acer negundo* L., *Ulmus pumila* L. is present in this area, *Juglans regia* L.

**Section No 2.** Primitive sedimentary undeveloped soils.

A, 0-10 cm – brown, relatively compacted, fine-grained, dryish. Rockiness – 5%. Densely permeated with plant roots.

C – dark grey, metamorphosed shale, lamellar, dry, penetrated by plant roots. Rockiness – 30%. Traced to a depth of 30 cm.

**Monitoring site No 3.** Lenin mine dump slope with southern exposure (Gornyatsky district, Makeevka). *Echium vulgare* dominates; *Picris hieracioides* L., *Senecio vernalis, Linaria maeotica*, *Reseda lutea* L., *Oberna behen* (L.) Ikonn. are also represented; among the woody plants in the area surrounding the site, *Robinia pseudoacacia*, *Acer negundo, Juglans regia* are found sporadically in the sample site.

**Section No 3.** Substrate with signs of soil formation.

A, 0-15 cm – brown, loose, fine-grained, dryish. Rockiness – 5%. The transition to the C horizon is gradual, with streaks in color.

C – fawn, traced to a depth of 30 cm. Rockiness – 15%. Salt efflorescence and shale metamorphization products are present.

**Monitoring site No 4.** Slope of the dump of mine № 12 "Naklonnaya" of eastern exposure (Proletarsky district, Donetsk). Monospecific group *Oberna behen*. The total projective coverage is 10-15%.

**Section No 4.** Primitive undeveloped fragmentary soils.

A, 0-27 cm – dry, dark grey, homogeneous, structureless, powdery, loose. No new growths are noted, stone content is 10%. The transition to horizon C is unclear in color.

C – dry, dark brown, homogeneous, structureless, powdery, loose. No new growths are noted, stone content is 50%. Sporadic roots. Traced to a depth of 60 cm.

In the profile, primary aggregation along plant roots is observed; the accumulation of humus has no morphological expression due to the weak development of the clay component. The existing humus is "camouflaged" by the grey color of the crushed rock.

**Monitoring site No 5.** The slope of the dump of mine № 12 "Naklonnaya" of northern exposure (Proletarsky district, Donetsk). The total projective coverage is 25-30%. Quite a lot of *Echium vulgare*, Oenothera biennis L., *Ambrosia artemisiifolia, Artemisia absinthium, Daucus carota, Centaurea diffusa, Linaria genistifolia* (L.) Mill., *Holosteum umbellatum* and *Senecio vernalis* grow scatteredly, *Rumex crispus* L. and annual shoots of *Acer negundo* are found sporadically. *Achillea pannonica* and *Chondrilla juncea* L. are added to the lower part of the site.

**Section No 5.** Primitive undeveloped soils.

A – 0-20 cm. Fresh, dark brown, homogeneous, structureless, powdery, loose. No new growths are noted, stone content is 25%. Densely permeated with plant roots. The transition to horizon C is clear in color.

C – dryish, dark grey, homogeneous, structureless, powdery, loose. No new growths are noted, stone content is 40%. Sporadic roots. Traced to a depth of 45 cm.

Determination of urease activity was carried out according to K.Sh. Kazeev (Kazeev et al., 2003). Statistical processing of experimental data was carried out using generally accepted methods of parametric statistics at a 5% significance level (Pryseds'kyj, 1999).

## RESULTS AND DISCUSSIONS

Analysis of urease activity in edaphotopes of anthropogenically transformed ecosystems in the spring period of research showed an ambiguous picture of its distribution along the soil profile. Thus, in the genetic horizons of the slope of the

Lenin mine dump (sites No 2-3), the urease activity in the parent rock was 1.5-3 times higher than the similar indicators of the humus-accumulative horizon (Table 1).

Table 1. Urease activity (mg $NH_3$/10 g soil per day) in soils of monitoring sites in spring

| Site, horizon | Non-reclamed | | Reclamed | |
|---|---|---|---|---|
| | M±m | % | M±m | % |
| № 2 Ao | 0.65±0.09* | 30.7 | 0.89±0.06* | 42.0 |
| № 2 Co | 1.98±0.08* | 618.8 | 2.63±0.03* | 821.9 |
| № 2 Ak | 0.65±0.09* | 30.7 | 0.74±0.04* | 34.9 |
| № 2 Ck | 1.98±0.08* | 618.8 | 2.21±0.03* | 690.6 |
| № 3Ao | 0.68±0.09* | 32.1 | 0.88±0.07* | 41.5 |
| № 3 Co | 1.04±0.23* | 325.0 | 1.39±0.06* | 434.4 |
| № 3 Ak | 0.68±0.09* | 32.1 | 0.77±0.05* | 36.3 |
| № 3 Ck | 1.04±0.23* | 325.0 | 1.15±0.06* | 359.4 |
| № 4 Ao | 0.45±0.06* | 21.2 | 0.61±0.05* | 28.8 |
| № 4 Co | 0.24±0.05 | 75.0 | 0.31±0.07 | 96.9 |
| № 4 Ak | 0.45±0.06* | 21.2 | 0.52±0.05* | 24.5 |
| № 4 Ck | 0.24±0.05 | 75.0 | 0.27±0.08 | 84.4 |
| № 5 Ao | 1.90±0.04* | 89.6 | 2.56±0.03* | 120.8 |
| № 5 Co | 1.90±0.10* | 593.8 | 2.43±0.08* | 759.4 |
| № 5 Ak | 1.90±0.04* | 89.6 | 2.18±0.11 | 102.8 |
| № 5 Ck | 1.90±0.10* | 593.8 | 2.15±0.09* | 671.9 |
| №1 A | 2.12±0.03 | | | |
| № 1 C | 0.32±0.03 | | | |

Here and in Tables 2–3: % - is the percentage of values exceeding those of similar soil horizons in site No 1, * - the differences are statistically significant at $p < 0.05$, o – plantings of *Onobrychis arenaria*, k – plantings of *Kitaibelia vitifolia*, % – percentage of excess

In the technozems of the remaining monitoring sites, the predominant trend was the intensification of enzymatic processes of ammonium accumulation within the upper horizon, leading in some cases to the achievement of enzymatic activity values in zonal soil. This fact can be explained by the extremely weak development of the upper soil horizon, the degree of formation of the phytocenosis and microbiocenosis, as well as the characteristics of the rocks that form these technogenic elements. The shortened soil profile and the relatively high biological activity of the underlying layer also cause an excess of urease activity in the C horizon to be 3-8 times higher in most monitoring sites compared to similar indicators in zonal soil (Table 1).

Sowing various crops at the monitoring sites had a positive effect on the processes of transformation of nitrogen compounds, which, in particular, can be judged by the level of enzymatic activity. Thus, the use of *Onobrychis arenaria* in the experiment led to an intensification of urease functioning in spring by 29-36%

compared to similar genetic horizons in areas with natural vegetation (Table 1). This established fact may be due to the fact that legumes have an effectively functioning rhizosphere symbiosis with nodule bacteria that actively absorb atmospheric nitrogen. A significant excess of the control level in the C horizon can be explained by the fact that the primitive soils of the studied monitoring sites have an underdeveloped soil profile of low thickness, poorly differentiated into horizons. When *Kitaibelia vitifolia* was used as a phytomeliorant, the degree of severity of its positive effect on the functioning of the studied enzyme was less pronounced, amounting to 11-16% compared to areas with natural vegetation cover. Along with this, the distribution of urease activity over the soil horizons of the monitoring plots was practically no different from the experimental variants in plots without sowing plants (Table 1).

In summer, we noted a statistically significant decrease in urease activity compared to the values obtained in the spring. Thus, in areas with natural vegetation, urease activity decreased by 27-70% in horizon A and by 17-68% in horizon C (Table 2).

Table 2. Urease activity (mg $NH_3$/10 g soil per day) in soils of monitoring sites in summer

| Site, horizon | Non-reclamed | | Reclamed | |
|---|---|---|---|---|
| | M±m | % | M±m | % |
| № 2 Ao | 0.37±0.03* | 23.0 | 0.49±0.05* | 30.4 |
| № 2 Co | 0.43±0.07* | 45.7 | 0.57±0.06* | 60.6 |
| № 2 Ak | 0.37±0.03* | 23.0 | 0.42±0.05* | 26.1 |
| № 2 Ck | 0.43±0.07* | 45.7 | 0.47±0.05* | 50.0 |
| № 3Ao | 0.56±0.03* | 34.8 | 0.77±0.08* | 47.8 |
| № 3 Co | 0.63±0.05* | 67.0 | 0.81±0.05 | 86.2 |
| № 3 Ak | 0.56±0.03* | 34.8 | 0.64±0.03* | 39.8 |
| № 3 Ck | 0.63±0.05* | 67.0 | 0.69±0.04* | 73.4 |
| № 4 Ao | 0.40±0.04* | 24.8 | 0.53±0.03* | 32.9 |
| № 4 Co | 0.30±0.05* | 31.9 | 0.39±0.03* | 41.5 |
| № 4 Ak | 0.40±0.04* | 24.8 | 0.46±0.05* | 28.6 |
| № 4 Ck | 0.30±0.05* | 31.9 | 0.33±0.05* | 35.1 |
| № 5 Ao | 0.33±0.04* | 20.5 | 0.42±0.03* | 26.1 |
| № 5 Co | 0.20±0.03* | 21.3 | 0.26±0.04* | 27.7 |
| № 5 Ak | 0.33±0.04* | 20.5 | 0.37±0.03* | 23.0 |
| № 5 Ck | 0.20±0.03* | 21.3 | 0.23±0.02* | 24.5 |
| № 1 A | 1.61±0.08 | | | |
| № 1 C | 0.94±0.07 | | | |

A similar trend was noted in the genetic horizons of model plots where experimental planting was carried out. In our opinion, the data obtained can be explained not only by more severe edapho-climatic conditions during this period of research, but also by a decrease in the activity of microbiological transformation of organic matter with the release of nitrogen-containing compounds and their active absorption by plants during the growing season. The highest phytoremediation effect was observed in monitoring areas with *Onobrychis arenaria seeded*, which was expressed in an increase in urease activity by 27-38% relative to areas with natural vegetation cover. Along with this, when using *Kitaibelia vitifolia*, not only a minimal positive effect on enzymatic activity was recorded (an increase of 9-15%), but also a decrease in its relative values in similar experimental variants compared to the spring period.

During the autumn period of research, a restoration of urease activity values to the level noted during spring sampling was recorded in the genetic horizons of soils of almost all studied monitoring sites (Table 3).

Table 3. Urease activity (mg $NH_3$/10 g soil per day) in soils of monitoring sites in autumn

| Site, horizon | Non-reclamed | | Reclamed | |
|---|---|---|---|---|
| | M±m | % | M±m | % |
| № 2 Ao | 0.60±0.05* | 30.3 | 0.71±0.06* | 35.9 |
| № 2 Co | 1.89±0.09* | 343.6 | 2.00±0.03* | 363.6 |
| № 2 Ak | 0.60±0.05* | 30.3 | 0.55±0.04* | 27.8 |
| № 2 Ck | 1.89±0.09* | 343.6 | 2.05±0.03* | 372.7 |
| № 3Ao | 0.63±0.03* | 31.8 | 0.59±0.07* | 29.8 |
| № 3 Co | 0.95±0.11* | 172.7 | 1.13±0.06* | 205.5 |
| № 3 Ak | 0.63±0.03* | 31.8 | 0.77±0.05* | 38.9 |
| № 3 Ck | 0.95±0.11* | 172.7 | 1.15±0.06* | 209.1 |
| № 4 Ao | 0.43±0.03* | 21.7 | 0.61±0.05* | 30.8 |
| № 4 Co | 0.31±0.07* | 56.4 | 0.31±0.07* | 56.4 |
| № 4 Ak | 0.43±0.03* | 21.7 | 0.52±0.05* | 26.3 |
| № 4 Ck | 0.31±0.07* | 56.4 | 0.27±0.08* | 49.1 |
| № 5 Ao | 0.85±0.05* | 42.9 | 1.56±0.03* | 129.3 |
| № 5 Co | 0.70±0.13 | 127.3 | 1.43±0.08* | 441.9 |
| № 5 Ak | 0.85±0.05* | 42.9 | 1.18±0.11 | 110.1 |
| № 5 Ck | 0.70±0.13 | 127.3 | 1.15±0.09* | 390.9 |
| №1 A | 1.98±0.05 | | | |
| №1 C | 0.55±0.09 | | | |

However, in the soils of site No 5, the noted trend was not so pronounced, which, in our opinion, is due to harsh hydrothermal conditions and underdeveloped plant communities (monospecific group *Oberna behen*).

The sowing of phytomeliorants had a beneficial effect on changes in the activity of urease in the soils of almost all genetic horizons of the monitoring sites. Maximum values of enzymatic activity, exceeding similar values in most areas with natural vegetation cover by 19-104%, were noted in variants of experiments using *Onobrychis arenaria*.

## CONCLUSIONS

Analysis of the data obtained showed that urease is confined to underlying genetic horizons, which is explained by the shortening of the soil profile and the leaching of soluble ammonium salts from the upper horizons. The study of the seasonal dynamics of the intensity of enzyme functioning showed a parabolic nature of changes in enzymatic activity with a minimum in the summer period of research and a maximum in the spring, during the period of enrichment of the soil with organic residues of the previous growing season. Sowing plants in monitoring areas had a positive effect on the processes of initial soil formation. Thus, when using *Onobrychis arenaria*, urease activity increased on average by 18-104% due to the fixation of atmospheric nitrogen by legumes, while in the variants of experiments using *Kitaibelia vitifolia* its activity increased by only 9-64%.

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