ҐРУНТИ ТЕХНОГЕННИХ ЛАНДШАФТІВ

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I. V. Kostenko, N. E. Opanasenko, M. L. Novitsky

APPLYING OF BIOASSAY TO THE ASSESSMENT OF SULFIDE MINE WASTES, SUBSTRATES AND YOUNG MINESOILS TOXICITY EVOLUTION

Nikita Botanical Gardens – National Scientific Center

Sulfide mine wastes dumped in the West Donbass region as well as technogenic substrates and young minesoils are characterized with the high variability of pH (2.94–9.00), exchangeable Al (0–770 mg kg^{-1}), water-soluble Mn (0–50 mg kg^{-1}), Na (9–980 mg kg^{-1}), sulfates (2–196 me kg^{-1}) and extractable carbon (0.012–0.886 %) concentration. A bioassay was used to study an influence of these soil conditions upon growth of wheat (*Triticum aestivum* L.). Is has been determined that under strongly acid conditions (pH<4.5) in leached soils, plant growth is suppressed by exchangeable Al, but the toxic effect of high Al in minesoils obtained from revegetated sites could be partly alleviated by humic acid accumulated there. In unleached samples, except Al, the water-soluble Mn and Na can occur in high concentrations that strengthens the toxic properties of minesoils. Low in Al, soil samples (pH 4.5–6.0) usually contain toxic levels of Mn and Na. The only factor of toxicity that has been found in neutral soil samples (pH>6.0) was the high concentration of soluble chlorides, NaCl mainly. So, the highest toxicity is inherent to recently dumped mine wastes and to the unleached ones in which all of the mentioned factors of toxicity are present simultaneously. In contrast, mine sites leached out of soluble salts despite of high acidity and exchangeable Al concentration reveal much lower toxicity and may be successfully revegetated with acid-resistant plant species.

Key words: sulphide mane wastes, substrates, minesoils, toxicity, bioassay.

І. В. Костенко, М. Є. Опанасенко, М. Л. Новицький

Нікітський ботанічний сад – Національний науковий центр

ВИКОРИСТАННЯ БІОТЕСТУ ДЛЯ ОЦІНКИ ЕВОЛЮЦІЇ ТОКСИЧНОСТІ СУЛЬФІДНИХ ШАХТНИХ ПОРІД, СУБСТРАТІВ І МОЛОДИХ ШАХТНИХ ҐРУНТІВ

Сульфідні породи шахтних відвалів Західного Донбасу, а також техногенні субстрати та молоді грунти, що формуються після їхнього окислення і подальшої трансформації, характеризувалися дуже широким спектром варіювання pH (2.94–9.00), вмісту обмінного Al (0–770 мг $кг^{-1}$), водорозчинного Mn (0–50 мг $кг^{-1}$), Na (9–980 мг $кг^{-1}$) та сульфатів (2–196 мг-екв $кг^{-1}$), а також вуглецю гумусових речовин (0.012–0.886 %). Вплив цих умов на рослини вивчалось за допомогою біотесту, в ході якого насіння пшениці (*Triticum aestivum* L.) пророщувалося в чашках Петрі на зразках шахтних порід, субстратів і молодих грунтів. Встановлено, що найбільш значний негативний вплив на зростання рослин в дуже сильнокислих умовах (pH<4.5) на вилугуваних зразках молодих ґрунтів спричиняє обмінний Al, однак його токсичність може бути частково нейтралізована гуміновими кислотами, накопиченими в процесі заростання відвалів. На сильнокислих і засолених субстратах на рослини негативно впливають також високі концентрації Mn та Na, що посилюють токсичність шахтних ґрунтів. В слабокислих зразках (pH 4.5–6.0) головним несприятливим для рослин фактором можуть бути високі концентрації водороз-

чинних сульфатів Mn та Na, а в нейтральних і лужних – хлоридів, головним чином NaCl. Таким чином, найбільша токсичність притаманна нещодавно відсипаним шахтним породам та шахтним субстратам, з яких не були вилугувані водорозчинні продукти окислення сульфідів. В той же час субстрати та молоді ґрунти з вилугуваних ділянок відвалів характеризувалися значно меншою токсичністю, що дозволяє проводити їх біологічну рекультивацію з використанням найбільш стійких до подібних умов рослин-ацидофілів.
Ключові слова: сульфідні шахтні породи, субстрати, ґрунти, токсичність, біотест.

И. В. Костенко, Н. Е. Опанасенко, М. Л. Новицкий
Никитский ботанический сад – Национальный научный центр

ПРИМЕНЕНИЕ БИОТЕСТА ДЛЯ ОЦЕНКИ ЭВОЛЮЦИИ ТОКСИЧНОСТИ СУЛЬФИДНЫХ ШАХТНЫХ ПОРОД, СУБСТРАТОВ И МОЛОДЫХ ШАХТНЫХ ПОЧВ

Сульфидные породы шахтных отвалов Западного Донбасса, а также формирующиеся после их окисления и дальнейшей трансформации техногенные субстраты и молодые почвы характеризовались очень широким спектром варьирования pH (2.94–9.00), содержания обменного Al (0–770 мг $кг^{-1}$), водорастворимых Mn (0–50 мг $кг^{-1}$), Na (9–980 мг $кг^{-1}$) и сульфатов (2–196 мг-экв $кг^{-1}$), а также углерода гумусовых веществ (0.012–0.886 %). Влияние этих условий на растения изучалось при помощи биотеста, в ходе которого семена пшеницы (*Triticum aestivum* L.) проращивались в чашках Петри на образцах пород, субстратов и молодых почв. Установлено, что рост растений в очень сильнокислых условиях (pH<4.5) на выщелоченных образцах молодых почв подвержен неблагоприятному влиянию обменного Al, однако его токсичность может быть частично нейтрализована гуминовыми кислотами, накопленными в процессе зарастания отвалов. На сильнокислых и засоленных субстратах токсичное влияние Al усиливается высокими концентрациями водорастворимых сульфатов Mn и Na. В слабокислых субстратах (pH 4.5–6.0) основными неблагоприятными для растений факторами могут быть высокие концентрации водорастворимых сульфатов Mn и Na, а в нейтральных и щелочных – хлоридов, главным образом NaCl. Таким образом, наибольшая токсичность присуща недавно отсыпанным шахтным породам, а также шахтным субстратам, из которых не были выщелочены водорастворимые продукты окисления сульфидов. В то же время субстраты и молодые почвы с выщелоченных участков отвалов характеризовались значительно меньшей токсичностью, что позволяет проводить их биологическую рекультивацию с использованием наиболее устойчивых к таким условиям растений-ацидофилов.
Ключевые слова: сульфидные шахтные породы, субстраты, почвы, токсичность, биотест.

Coal mine wastes newly extracted to the surface are characterized by high skeleton contents, close to neutral pH and high soluble salts (mainly chlorides) concentration. Exposed to the atmosphere, these materials undergo the pyrite oxidation, decomposition of coarse fragments, acidification and mineral transformation. The rate of mine wastes properties' normalization and natural revegetation depends much on the peculiarities of dump surface's relief. It has been shown (Kostenko, 2005) that the accumulation of leached fine earth in the depressions between the hillocks at the unleveled surfaces of abandoned mine sites promotes the successions of plants that's accompanied with the gradual accumulation of humus and minesoils formation. The leveled surfaces as well as the slopes of hillocks remain open for decades due to unfavorable physical (high skeleton contents, high density, low water holding capacity) and chemical (extremely low or, on the contrary, extremely high pH and high soluble salts concentration) conditions.

The role of exchangeable Al in the toxicity of acid sulfate soils including minesoils is well-known. As these soils are exclusively the mineral soils which contain only traces of organic mater (besides of coal debris) in topsoil of the revegetated sites, the contribution of H-ions to the minesoils acidity are negligible and hardly detected. On our calculations the Al contributes up to 80–99 percents to the total minesoils acidity being therefor the main factor of these soils toxicity. These values are typical for the acid sulfate soils of Texas that was shown in the study of Carson (1983). The dominant forms of Al in acid conditions (pH<5) are the trivalent Al species (Delhaize, 1995), so all of the Al found in acid sulfate minesoils appears to be toxic for plants. Besides of exchangeable, the water-soluble Al in sulfates rich soil horizons was detected which influence on plant growth should also be studied. The toxicity of acid minesoils may be strengthened through the release of other than Al cations to soil solutions, among which Mn, Fe, Cu, Zn, and Ni are commonly mentioned (Barnhisel, 1969; Massey, 1972). It was found out in the preliminary researches that the acid minesoils release only negligible concentrations of Fe, Cu, and Zn to water extract and that the concentrations of these cations measured in ammonium-acetate buffer extract (pH=4.8) are similar to those of the most Ukrainian soils, so that these cations may be considered rather as nutrients than as toxicants. Among potential toxicants besides of Al, only water-soluble Na and Mn were found in the concentrations which theoretically can impede the growth of plants on acid sulfate minesoils under study. Manganese toxicity is a serious problem to many crops grown on acid soils when total Mn concentration greatly exceeds the average worldwide Mn concentration. In Hawaiian Oxisol total Mn varied from 10 to 40 g kg^{-1} (Hue, 2001, 2002) and the concentration of Mn in the saturated paste extract reached 72 mg L^{-1} at pH 4.25. To avoid Mn toxicity for the sensitive species (beans, lettuce, potato, and roses), it was proposed (Hue, 2001) to keep Mn concentration in the saturated paste extract below 0.5 mg L^{-1} that responds to about 0.5 mg kg^{-1} of soil. In soil Mn toxicity is alleviated by other nutrients, such as Ca, Mg, Si (Bot, 1990; Horst, 1978; Hue, 2001).

As for Na, no information is available on its behavior in acid sulfate minesoils and its influence on plant growth because of the active leaching of soluble salts under sufficient water supply and the precipitation of Na as water-insoluble natrojarosite ($NaFe_3(SO_4)_2(OH)_6$). Although Na is not commonly mentioned as a toxicant in acid minesoils, with regard to the possibilities of very young minesoils salinization it should be interesting to determine the level that the concentration of soluble Na could reach in different age mine sites and the toxic level of soil Na for plant growth.

The organic acids in acid soils produce the immobilizing effect on Al due to chelating of Al that makes it inactive and unavailable for reaction with P (Grenda, 2001, Haynes, 2001; Hue, 1986; Suthipradit, 1990; Tan, 1986). In contrast, the organic acids produce the mobilizing effect on Mn (Grenda, 2001). Applying of organic rich materials, such as cowpea green manure and biosolids, significantly impeded plant growth according to Hue et al. (2001). It was postulated that organic molecules could dissolve Mn oxides and maintain a high level of Mn in solution. Thus, the role of humic acid both of a geogenic and plant residues origin in alleviation of minesoils toxicity should be tested.

The bioassay is a wide-used method for the assessment of Al soil toxicity and Ca deficiency in acid soils (Ahlrichs, 1990). It was important to examine the validity of this method to discover the toxicity of other soil factors in minesoil.

The objective of this study is to find out and quantify the main factors of the acid sulfate minesoils' toxicity in mine sites differing in age, relief and time since dumping, reveal the tendency of the minesoils' toxicity evolution so that to manage properly the detoxication of these lands to make them fit for low-cost revegetation.

MATERIALS AND METHODS

A set consisting of 185 samples of acid sulfate substrates and minesoils obtained from the surface of coal wastes dumps was selected for this study. Soil samples were collected from 0 to 0.4–1.2 m depth on unleveled dump surfaces at revegetated with herbaceous species or non-vegetated depressions, and at non-vegetated slopes of hillocks. The age of the dump surfaces studied varied from 15 to 20 years. Other soil samples were taken from the recently dumped (1–3 years) and leveled mine sites. Newly extracted mine wastes were sampled in day of dumping.

Samples were air dried, ground and sieved to the size <1mm. Soil pH was measured in a 1M KCl solution at 1:2.5 soil/solution ratio by weight. Exchangeable Al (1M KCl extract at 1:25 soil/solution ratio) as well as water-soluble Al species (1:10 soil/water ratio) after shaking for 1 h. were determined colorimetrically with xylene orange. In water extract were measured also Mn and Na concentrations by atomic absorption spectrophotometry and SO_4^{2-} by conductimetric analysis. Since the direct determination of humus in coal rich minesoils through the measuring of C-total is impossible, the following procedure was used for the humus substances' determination: humic acids were extracted with the mixture of 0.1N NaOH and 0.1M $Na_4P_2O_7 \cdot 10H_2O$ (pH=13) at 1:10 soil/solution ratio after 20 hours extraction period (Kononova, 1961) and measured colorimetrically after drying and digesting with the mixture of 0.4N $K_2Cr_2O_7$ and conc. H_2SO_4.

All of the samples analyzed were separated into leached and unleached according to the water-soluble sulfates concentration. To leached it were referred soil samples containing less than 3 me kg^{-1} of SO_4^{2-}.

Bioassay. Thirty uniformly sprouted seeds of wheat (*Triticum aestivum* L.), after having passed 24h-germination period at 25 °C, were planted in the glass petry dish filled with 20 g of soil. Before planting, soil was carefully moistened with 10 ml of distilled water and equilibrated for 5 hours. Seeds were uniformly distributed on the soil surface and covered with lids. After incubation in thermostat at 23 °C for 72 h, the sprouts were carefully cut off and immediately weighted. The total fresh weight of sprouts (TFWS) obtained for every dish was used as an index of plant growth. A filter paper placed in dish and moistened with 5 ml of distilled water was used as a control. Such a control was included in every experiment comprising of 20–25 dishes. To make the results comparable, the TFWS was expressed in percentage of the control. As a result, the relative sprouts weight (RSW) was obtained and used for the further statistical calculation (STATISTICA 5 software). The variability of RSW for five replications did not exceed 7 %.

Subsequent experiment 1. To determine the critical level of Na in soil solution, a special bioassay experiment for discovering Na toxicity has been conducted. For this experiment, two soil sample with different chemical characteristics were chosen. First of them was acid (pH=3.48), high in exchangeable Al (276 mg kg^{-1}), and high in extractable C (0.226 %). The second soil sample was neutral (pH=6.72) and about twice as low in extractable C (0.114 %). Every sample was divided into six subsamples (1.0–1.5 and 2.0–2.5), placed in a petry dish and moistened with increasing concentrations of NaCl (Table 3). At the end of the experiment, soil samples were analyzed for pH, water-soluble Na and Mn.

Subsequent experiment 2. To answer the question: Whether the water-soluble Al appearing in soil solution of gypsum-rich acid sulfate minesoils is or not toxic for plant, we took soil samples of topsoil (pH=3.48; Al exch.=202 mg kg^{-1}; Cextr.= 0.226%) and subsoil (pH=3.44; Al exch.=142 mg kg^{-1}; Cextr.=0.066%). These samples were divided into subsamples and thoroughly mixed with gypsum (Table 4) prior to be placed into petry dishes and planted as described above.

RESULTS AND DISCUSSION

Minesoils properties. Consisting of shales, sandstones, and pyritic coal debris and being exposed to the atmosphere, mine wastes undergo oxidation, mineral transformation, leaching of soluble products, fine earth accumulation, and nutrient status formation so that they ultimately become a medium suitable for plants. So, the properties of minesoils under study mainly depend on the time of dumping, relief, soil density and the rate of revegetation. Freshly exposed to the atmosphere mine wastes are neutral, very high in water-soluble Na as Na_2SO_4 and NaCl, free of mobile Mn and Al, and very low in extractable C (Table 1).

Being compacted through the leveling by caterpillars, recently dumped mine wastes were mostly high both in acidity and soluble salts concentration, but the ratio between the soluble compounds varied depending on the time since dumping and the depth. The soluble compounds distribution through the profile is not yet typical to the more or less developed minesoils, thus their concentration in the top 0–20 cm horizon often exceeds that in deeper layers (Table 1). The concentration of extractable carbon of humic substances usually varies from 0.02 to 0.06 % except for one site with ten times higher C concentration (0.47 %). Although during some time after dumping the concentrations of Na gradually decreased through leaching and the precipitating of natrojarosite, most of the samples obtained from recently dumped and leveled mine wastes remained high in soluble Na (Table 1). Unlike Na, Mn reaches its highest concentrations in recently dumped wastes because the Mn released through Mn oxides destroying by sulfuric acid is not leached out of soil profile non the less.

Naturally revegetated depressions between the hillocks are characterized by the highest leaching of the soluble salt with additional runoff from the adjacent slopes (Table 1). Besides, the runoff removes the leached fine earth from the slopes and accumulates it at the depressions, so that the skeleton contents of these sediments in subsoil (10–30 %) become much lower than that of the subsoil and of the adjacent slopes (70–90 %). The pH of these soils varied greatly, but the concentration of the water-soluble toxicants (Na, Mn) was very low. The chemical and physical properties of the revegetated depressions topsoil suite those plant species which are acidophilic and tolerant to low fertility. The decomposition of plant litter leads to the formation of humus horizon that has the extractable carbon concentrations several times higher than that for the non-vegetated sites.

Table 1

Mean values of the selected properties of acid sulfate minesoils and RSW of wheat
(Al exchangeable, Mn and Na water-soluble)

Horizon	Number of samples	pH		C extr.	Al	Mn	Na	RSW
		min-max	mean	%	mg kg^{-1}			%
Newly extracted mine wastes								
–	6	6.45–7.00	6.59	0.023±0.002	0	0	963±25	80±5
Recently dumped (1-3 yr.) and leveled mine wastes								
Topsoil	20	3.25–8.00	3.67	0.095±0.108	150±180	20±25	271±267	62±42
Subsoil	24	2.98–7.05	3.45	0.136±0.170	228±230	23±17	195±236	48±42
Revegetated depressions								
Topsoil	38	3.00–8.00	3.38	0.172±0.141	205±173	1.9±2.6	19±9	94±31
Subsoil	30	2.94–7.78	3.64	0.116±0.093	232±184	4.8±6.2	23±12	80±34
Non-vegetated depressions								
Topsoil	3	3.08–3.37	3.22	0.073±0.041	464±93	1.5±0.5	27±2	37±11
Subsoil	3	3.15–3.25	3.18	0.045±0.018	384±50	0.6±0.2	23±3	37±7
Slopes of hillocks								
Topsoil	28	2.94–6.31	3.43	0.037±0.017	201±203	8±10	37±67	74±42
Subsoil	33	2.96–6.70	3.54	0.079±0.043	211±271	8±10	31±49	67±38

Being low in skeleton and leaching just as the minesoils of revegetated depressions do, the minesoils of non-vegetated depressions also remain unsuitable for plants for long enough period mainly because of the highest both acidity and exchangeable Al concentration among the studied minesoils (Table 1).

The slopes of hillocks are characterized by the high variability in pH and much lower leaching of soluble salts among which the gypsum is dominant (Table 1). The topsoil concentration of the water-soluble Na and Mn, as compared with the revegetated depressions, was more than twice and more than seven times higher, respectively. The rate of these soils revegetation and humus accumulation is very low.

Not only the exchangeable Al which is typical for acid soils at pH<4.5, the water-soluble Al was detected as well. Most often, the latter was found in unleached soils samples, for which the Al maximum concentration reached 452 mg kg^{-1} or 49 percents of the total amount of Al extracted with 1M KCl. In leached minesoils, in spite of the exchangeable Al high contents, the concentration of water-soluble Al did not exceed 0.5–1.0 mg kg^{-1}. Analysis of these data reveals the multiple correlation of the water-soluble Al to the concentrations of water-soluble sulfates (mainly gypsum) and the exchangeable Al (R=0.90***; n=43) presented at Figure 1, that enabled us to postulate that the water-soluble Al is the Al substituted with Ca from gypsum in the water suspension. Nevertheless, we do not exclude the possibility of aluminum sulfates precipitation in gypsum-rich soil horizons as those observed by Schaaf et al. (1999) in minesoils of the Lusatian lignite mining areas.

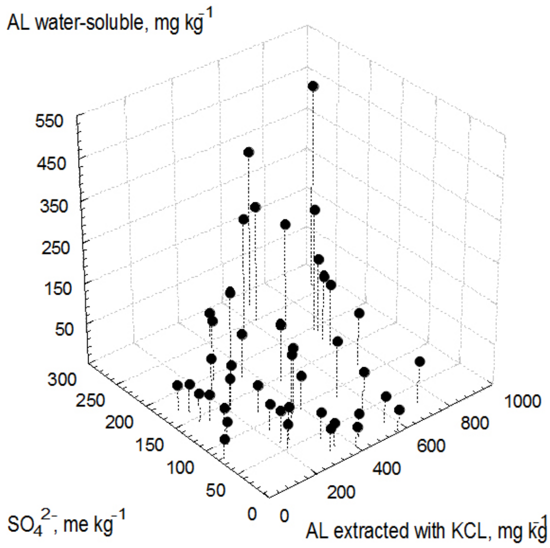

Fig. 1. **Concentration of water-soluble Al in acid sulfate minesoils as a function of Al extracted with 1M KCl and water-soluble sulfates concentration**

A high water-soluble Mn concentration in minesoils was associated with low pH (Figure 2), but this relation was not so definite as those obtained by Hue et al. (2001) and Hue and Mai (2002) in Mn rich Hawaiian Oxisol. One can see that pH controls the upper level of Mn solubility in unleached samples, whereas the soil samples leached out of the soluble salts have a very low Mn concentration regardless of pH. So, we can postulate that water-soluble Mn occurs as $MnSO_4$ precipitating in acid sulfate soils due to Mn oxides being destroyed by sulfuric acid that resulted in relation of water-soluble Mn to the concentration of water-soluble sulfates (r=0.62***; n=176). Easily soluble, this Mn is leaching out of soil profile under sufficient water supply so that even highly acid minesoils often contain only negligible concentrations of soluble Mn.

Results of bioassay. The bioassay reveal the tendency for the toxicity to lower in the topsoil horizons relatively to the subsoil with except of the non-vegetated depressions where both the topsoil and also subsoil horizons are extremely high in exchangeable Al, and the tendency for the toxicity to get lowest level in minesoil of the revegetated depressions as compared with the non-vegetated slopes of hillocks and the recently dumped mine sites in spite of them having close mean pH and exchangeable Al values (Table 1). As the sites mainly differ in their rates of leaching, the RSW obtained for leached and unleached soil samples were separately plotted against pH (Figure 3). It is obvious, that the RSW obtained for the soil samples, which are free of soluble salts and relatively high in humus, could exceed 100 % (sprouts weight of control) at pH=3.25 and higher in spite of high acidity and exchangeable Al high concentration. At pH 4.2–4.5, RSW for these samples reached the highest values and the further pH increasing did not influence wheat growth in our experiment. In unleached and humus-free samples of minesoils, the RSW reached the highest values only at pH about 5.0–5.25 when the concentration of exchangeable Al goes down to 0. A large data scattering testifies the presence of some other soil factors except of acidity and Al, that influence wheat growth as well. Partial and multiple correlation (Table 2) reveal the significant influence of the exchangeable Al, water-soluble Mn, Na, and extractable C concentration on RSW.

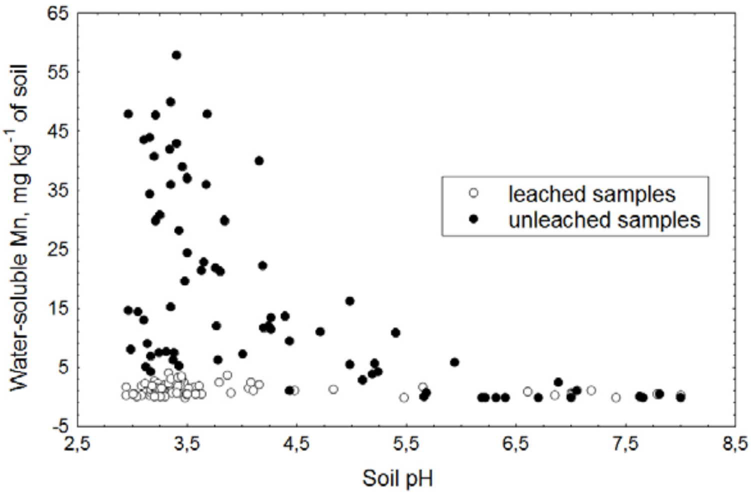

Fig. 2. **Concentration of Mn in acid sulfate minesoils as a function of soil pH**

Table 2

Partial and multiple correlation coefficients for RSW and soil factors studied

RSW	n	Partial correlation with				Multiple correlation
		Al	Mn	Na	C_{extr}	
Total	185	-0,77***	-0,49***	-0,17*	0,26***	0,83***
Leached samples	80	-0.87***	-0.24*	NS	0,35***	0.89***
Unleached samples	105	-0,76***	-0,41***	NS	NS	0,82***

NS – non significant at p-level <0.05

As it could be expected, the plant growth was inhibited by the exchangeable Al (soil acidity) mainly, that‘s followed by water-soluble Mn and Na. Humic acid positively influenced the growth of wheat. The separate multiple regression for leached and unleached soil samples reveals the exchangeable Al and the water-soluble Mn to be the common factors of minesoils toxicity peculiar to all soils studied at low pH (Table 2). However, the Mn negative influence on the plant growth in the unleached soil samples was much significant as compared with that in the leached samples. Humic acids promote plant growth in the leached soil samples from depressions. The Na negative influence on the plants growth for unleached soil samples was non significant. These results confirmed the well known toxic effects of Al and Mn on plant growth in acid environment as well as the positive effect of organic acids in aluminum toxicity's alleviation (Suthipradit, 1990). Na is not generally considered to be potentially toxic element in acid sulfate minesoils due to the reasons mentioned above. In a droughty zone (annual precipitation 477 mm) where leaching is retarded, soluble Na can remain in soil profile for a long time along with Al and Mn. Although Na was recognized as a possible toxicant in bioassay study, the level of Na toxicity remains yet unknown. Through the subsequent experiment 1 it was found that despite of the fact that both of the sample groups were treated with the same concentrations of NaCl, the soil Na concentrations found in the first sample group were higher than in the second one, that may be explained by their difference in soil's buffering capacity. Treating the soils with NaCl promoted the exchangeable Mn releasing to solution and gradual lowering of pH in acid soil samples, whereas in neutral soil samples the pH lowering turned into growing when concentration of Na reached 790 mg kg^{-1}. Plants response to NaCl adding depended on minesoils properties. Under acid conditions, the RSW has fallen below 90 % relatively to the untreated variant when the concentration of Na in a soil solution exceeded 195 mg kg^{-1} due to the combined influence of soluble Na and exchangeable Al on plant growth. Under neutral soil conditions, RSW has fallen below 90% relatively to the untreated variant when the concentration of Na in a soil solution exceeded 250 mg kg^{-1} (Table 3).

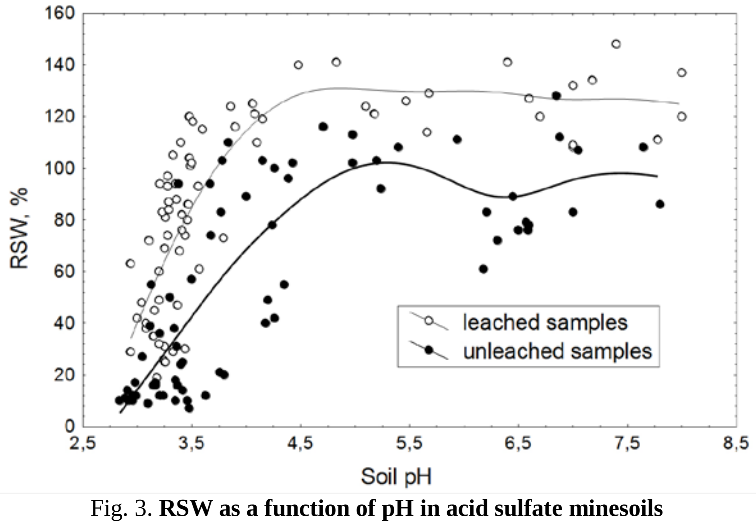

Fig. 3. **RSW as a function of pH in acid sulfate minesoils**

Table 3

Results of the experiment with NaCl added to minesoils

Sample	Na added	Na H_2O found	Mn H_2O	pH H_2O	RSW as compared with untreated variants
	mg kg^{-1}				%
1.0	0	42	0	4.06	100
1.1	200	195	0.9	4.23	94
1.2	390	360	0.9	4.18	71
1.3	790	690	1.8	4.15	40
1.4	1570	1240	2.8	4.01	17
1.5	3150	2680	2.8	4.00	2.3
2.0	0	34	0	6.87	100
2.1	200	99	0	6.84	95
2.2	390	250	0.4	6.72	93
2.3	790	530	0.7	6.75	76
2.4	1570	1160	1.3	6.82	47
2.5	3150	2350	2.0	6.90	8

The lower toxicity of Na in neutral soils responds to a much lower plant Na concentration for 1570 and 3150 mg kg^{-1} of Na applied as compared to acid soils (Figure 4), that is evidently connected with better supply of root medium with Ca in neutral soil containing 10 meq $100g^{-1}$ of exchangeable Ca that is 3.5 meq $100g^{-1}$ higher than in acid soil. Kinraide (1998) suggested the importance of Ca in alleviation of H, Al, and Na soil toxicity in wheat. So, we may conclude that Na (NaCl) toxicity gets evinced when the concentration of Na exceeds 200 mg kg^{-1} in the acid minesoils unsaturated with respect to Ca. This level is rarely exceeded in the topsoil of acid sulfate soils at the surfaces of the mine land sites abandoned over 10 years ago. For these lands, the main sources of soil toxicity are exchangeable Al and water-soluble Mn. The gradual accumulation of humus promotes the lowering of Al toxicity and enhance the plant diversity. The toxicity of Na (NaCl) in Al- and Mn-free neutral minesoils saturated with Ca evinces itself when the concentration of water-soluble Na exceeds 250 mg kg^{-1}. Nevertheless, even further increasing of Na concentration in this case does not produce such a negative effect on plant growth as on acid soils. Water-soluble Na concentrations exceeding the levels of toxicity are typical for as recently dumped so as newly extracted mine wastes.

Another question to be answered: Whether the water-soluble Al appearing in soil solution of gypsum-rich acid sulfate minesoils is or not toxic for plant? For this reason a subsequent experiment 2 was conducted. Applying of gypsum did not change pH, but resulted in drastic increasing of the water-soluble Al concentration (Table 4) according to the above-mentioned dependence of the soluble Al on the soluble sulfates and the exchangeable Al concentration . The concentration of water-soluble Mn has increased but to a less degree as compared with Al. Raising of the Al and Mn mobility has almost not changed RSW of wheat that confirms the importance of $CaSO_4$ in alleviation of Al toxicity through the formation of less phytotoxic $AlSO_4^+$ species (Noble, 1988).

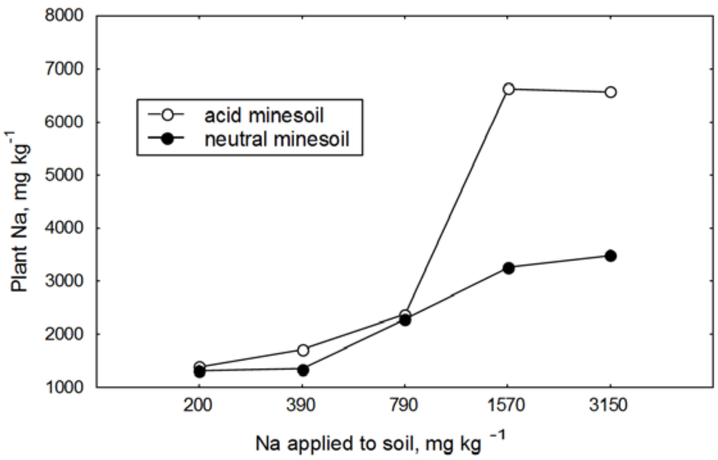

Fig. 4. **Na concentration in plant tissue as a function of Na applied to neutral and to acid minesoils**

Table 4

Results of the experiment with $CaSO_4 \cdot 2H_2O$ added to minesoils

Sample	Gypsum added	SO_4^{2-} H_2O found	Al H_2O	Mn H_2O	pH H_2O	RSW
	g kg^{-1}	me kg^{-1}	mg kg^{-1}			%
1.0	0	7	0	0	4.06	95
1.1	2.5	53	3.5	4.1	3.95	85
1.2	5.0	69	8.2	6.0	3.91	79
1.3	10.0	116	20.0	6.4	3.96	74
1.4	25.0	276	39.0	4.9	3.94	84
1.5	50.0	300	41.0	4.8	3.95	80
2.0	0	18	0	3.2	4.00	82
2.1	2.5	56	5.3	10.8	4.01	86
2.2	5.0	73	8.0	12.2	3.98	94
2.3	10.0	133	17.0	12.2	4.00	75
2.4	25.0	289	35.0	10.7	3.99	91
2.5	50.0	299	32.0	9.3	4.01	89

1.0-1.5 – samples of topsoils;
2.0-2.5 – samples of subsoil.

The results of the bioassay have been compared with the results of our previous field investigations on the natural revegetation of the abandoned mine sites (Kostenko, 1999, 2005). According to the field researches, plant growth starts in leached depressions if pH does not drop below 3.2. An average RSW obtained for highly acid samples of minesoils (pH 3.21–3.29, concentration of the exchangeable Al=282 kg^{-1}, $C_{extr.}$=0.192 %) from depressions was 89±6 % that permits us to accept this level of RSW as critical if the possibility of natural or artificial revegetation of the sulfide-containing mine wastes with acid-resistant plant species is to be considered. It means that any attempt to revegetate those minesoils for which the RSW obtained in bioassay does not exceed 85–90 % fails most likely.

CONCLUSION

The results of research have revealed three main factors of toxicity in acid sulfate minesoils studied: exchangeable Al, water-soluble Mn and Na. The occurrence of these soil factors depends on the initial wastes' pyrite concentration, time since dumping, dumping technique, and relief. In the range of pH from 2.96 to 4.5, the mobile forms of Al suppress plant growth in the leached soils of depressions; the mobile forms of Al and Mn suppress plant growth in the slopes of hillocks; the mobile forms of Al, Mn, and Na suppress plant growth in the soils of recently dumped and compacted mine wastes. In the range of pH from 4.5 to 6.0 no factors of toxicity with respect to wheat were found in the leached soils of depressions; the mobile forms of Mn were found in the slopes of hillocks; the mobile forms of Mn and Na were found in the soils of recently dumped and compacted mine wastes. At pH>6.0, plant growth may be suppressed by high soluble Na in the soils of recently dumped and compacted and newly extracted mine wastes as well.

Although many of soil samples obtained from the topsoil of the non-vegetated slopes of hillocks were low in toxicity, these sites do not revegetate mainly due to the unfavorable physical properties of their surfaces covered with the stony crust preventing seeds to fix and germinate. It means that bioassay applied is capable to discover the main chemical factors of acid minesoils toxicity, however to reveal another potentially adverse factors impeding the revegetation of mine lands the researches should be accompanied with field investigation of natural revegetation and physical properties of minesoils.

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І. Х. Узбек

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