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Ecotoxicological assessment of mineralized stratum water as an environmentally friendly substitute for agrochemicals

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Abstract. As a result of military operations on the territory of Ukraine, sown areas are reduced, the cost of plant protection products and fertilizers increases which emphasizes the problem of obtaining maximum yields from a smaller area of farmland. Given that a shortage of food grains can cause a global food crisis, research on the use of MSW as an environmentally friendly substitute for agrochemicals is relevant today. The aim of the research was to assess the ecotoxicological properties of MSW as an environmentally friendly substitute for synthetic agrochemicals. The impact of MSW as a fertilizer on soil chemical properties and assessment of MSW phytotoxicity as an herbicide for weeds and productivity of winter wheat were studied under field conditions. Toxicological assessment of MSW under laboratory conditions was carried out according to the following parameters: acute oral toxicity and resorptive-toxic effect of MSW. As a result of the assessment of MSW impact on soil chemical properties, it was found that significant soil acidification occurs only when MSW dose of more than 2,400 L ha^{-1} is used. The content of nitrates and oil products did not increase and there was no soil salinity when the MSW was used in doses of 300–1,200 L ha^{-1}. It was determined that the greatest decrease in weed plant biomass (85.5%) was observed when 100% concentration of MSW was used in a dose of 350 L ha^{-1}. As a consequence, with reduced weed infestation, there was a 21.5% increase in winter wheat yield if 100% MSW was used and a 19.1% increase if 75% MSW was used. As a result of the toxicological assessment of MSW, it was found that it belongs to low-toxic compounds. These results of ecotoxicological investigation of MSW make it possible to assert that its use is safe in agriculture, in particular as an environmentally safe organomineral fertilizer and herbicide.

Key words: ecotoxicological assessment, mineralized stratum water, soil, phytotoxicity, winter wheat.

INTRODUCTION

During the extraction of oil and gas, large quantities of mineralized stratum water (MSW), which is a by-product, come to the surface. The problem of disposal of large quantities of this water is very significant, given that uncontrolled release of large quantities of stratum water on the ground, leads to salinization and deterioration of the agronomic structure of the soil, the destruction of biodiversity in natural ecosystems. Based on the research conducted by (Obire & Amusan, 2003; Reva, 2016) it was found that MSW contains a significant amount of mineral elements and inorganic compounds (about 60 different micro- and macroelements), in particular sulfates and chlorides, the total mineralization is in the range of 140–180 g dm^{-3}. However, the impact of MSW in different doses on the soil has not been studied sufficiently.

The previous research conducted by Markina (2019) established the possibility of using MSW as an environmentally friendly substitute for agrochemicals on the crops of cereals in order to increase their yields. As a result of warfare in Ukraine, sown areas are being reduced, which raises the issue of maximizing the yield from smaller areas of farmland, as well as the protection of crops from weeds by inexpensive and environmentally safe means. This issue is especially important nowadays, since the lack of food grains can cause a global food crisis. Therefore, it is necessary to search for new approaches of using environmentally friendly and cost-effective plant protection agents in agriculture. The use of mineralized stratum water, which is a by-product of oil production, can become one of such methods.

The possibility of using MSW in order to improve the technology of obtaining high-quality organic fertilizers was determined earlier, Pisarenko et al. (2022). The phytosanitary impact of MSW on the crops of cereals was studied as well (Pysarenko, 2021). The problems of application of MSW as a substitute for agrochemicals, in particular the determination of environmentally safe doses of MSW as a herbicide and the main fertilizer of cereals, require further research.

According to the assesment of Pisarenko (2019), mineralized stratum water of oil and gas fields in Poltava region was evaluated as mineral therapeutic bromine, iodine-bromine sodium chloride, calcium-sodium brines, promising for balneotherapy and safe for people. In accordance with the Decree The Cabinet of Ministers of Ukraine, (1996), before using any new plant protection agents, it is necessary to conduct their toxicological assessment and determine their class of hazard for the environment, warm-blooded animals and bees. Therefore, before using MSW as a substitute for agrochemicals, it is necessary to assess its toxicity by LD_{50} in accordance with the resolution of the World health organization (Executive Committee, 55^{th} session, 1974).

The problem under study is pressing both for the oil and gas industry and for agricultural production. The aim of the scientific paper was to assess the ecotoxicological properties of MSW as an environmentally friendly substitute for synthetic agrochemicals. This envisaged the study of the impact of different doses of MSW on the agrochemical soil properties, assessment of MSW phytotoxicity to weeds, determination of winter wheat productivity under these conditions, as well as assessment of MSW toxicity in order to determine its hazard class.

MATERIALS AND METHODS

For the purpose of maximum and comprehensive study of MSW for use as agrochemicals, field and laboratory experiments were set up. Under field conditions, the impact of MSW as a fertilizer on soil chemical properties, assessment of MSW phytotoxicity as an herbicide for weeds and productivity of winter wheat were studied. To use any solutions as agrochemicals, it is necessary to assess their toxicity to the environment, warm-blooded animals and bees (Decree The Cabinet of Ministers of Ukraine, 1996). Therefore, the toxicological assessment of MSW under laboratory conditions was performed according to the following parameters: acute oral toxicity and resorptive-toxic effect of MSW.

At the first stage, the impact of MSW on soil chemical properties was studied. This research was conducted for 5 years (2017–2021) in three-fold repetition in the experimental fields of PSAU (Poltava State Agrarian University). The plots with an area of 0.5 ha were made (Trybel, 2001) MSW concentrations of 300 L ha^{-1}, 600 L ha^{-1}, 900 L ha^{-1}, 1,200 L ha^{-1}, 2,400 L ha^{-1} and 4,800 L ha^{-1} were applied on these plots as a fertilizer during the basic tillage. The plots without MSW and the plots where full mineral fertilizer $N_{50}P_{50}K_{50}$ was applied were taken as control.

Cultivated crop is winter wheat. The soil of the experimental field was typical deep low-humus medium-loam soil of Haplic Luvisol type (according to WRB, 2014): humus content - 3.6%, total nitrogen - 0.32%; hydrolytic acidity - 2.39 mg eq per 100 g, easily hydrolyzed nitrogen (N) - 141 mg kg^{-1} soil, P_2O_5 - 269 mg kg^{-1} soil, K_2O - 87 mg kg^{-1} soil. MSW was characterized by: pH 8.7–8.9, Na+K 45.8–50.2 g dm^{-3}, Ca^{2+} 10.9–11.1 g dm^{-3}, Mg^{2+} 0.9–1.0 g dm^{-3}, Cl^- 95.6–105.2 g dm^{-3}, SO_4^{2-} 6.8–7.0 g dm^{-3}, HCO_3^- 0.82–1.15 g dm^{-3}, the oil-hydrocarbon content - 3–5%.

Soil chemical properties were determined 30 days after MSW application by the following methods: actual soil acidity by measuring H^+ concentration in the solution by potentiometry method using ion-selective electrodes on a potentiometer of pH-150 M (ISO 10390:2021); the content of exchangeable sodium, macro- and microelements: potassium, calcium, magnesium in the aqueous extract by using a flame photometer with a wavelength of 589 and 569.9 nm (ISO 11047:1998); hydrocarbonates, carbonates (ISO 10693:1995), sulfates (ISO 11048:1995), chlorides, iodine, bromine and others were determined according to ISO 11260:2018. Petroleum products were determined by gravimetric method ISO 11504:2017.

At the second stage, the phytotoxicity of MSW to weeds in winter wheat crops was assessed by weight method under field conditions. For this purpose, the plots with an area of 0.5 ha were set up, where spraying was carried out during the period of winter wheat tillering. Consumption rate of MSW was 350 L ha^{-1}, when it was used as a herbicide (Trybel, 2001). The following experimental plots with a concentration of 100% MSW; 75% MSW; 50% MSW were examined. The comparison was made with the herbicide Desormone 600 (the main active ingredient - 2.4 dichlorophenoxyacetic acid in the form of dimethylamine salt, 600 g L^{-1}). On day 30 after treatment, soil weed control was carried out. All weeds were counted, uprooted, air-dried and weighed (Trybel, 2001). Part of the damaged surface was separated, weighed, and the percentage of leaf surface damage was measured. The experiment was conducted in three-fold repetition for 5 years.

On these experimental plots, where MSW was used as a herbicide, winter wheat yields were tested for 5 years. Winter wheat yield accounting was carried out by sheep sampling in 3-fold repetition on the accounting plots in the phase of full grain ripeness (Trybel, 2001).

In accordance with the Decree The Cabinet of Ministers of Ukraine (1996) before the beginning of state trials of new chemical plant protection agents, it is necessary to carry out their toxicological assessment, which will determine the class of hazard and develop measures for the safe use of the preparation.

Toxicological studies used white rats and white mice that have taken the 14-day quarantine in the vivarium of Poltava State Agrarian University according to the method of Menshikova (1987). Acute oral toxicity of MSW was studied on white rats of *Wistar* line and nonlinear white mice. The main criterion for the toxic effect was the dose that caused the death of 50% of the animals (LD_{50}). Six *Wistar* rats and 10 white mice were used to determine the mean lethal dose. Adult rats weighing 225–240 g were administered MSW at a rate of 21,300 mg kg^{-1} body weight. The preparation was administered in the native state. The fluid intake was 5 ml per rat. Adult white mice weighing 20–30 g were administered MSW at rates of 24,000 mg kg^{-1}, 34,000 and 36,000 mg kg^{-1}. The technique of administration of the preparation to the stomach was followed and the data on the amount of liquid allowed to the animals depending on the method of administration, their species and weight were taken into account (Sidorov, 1976) Within 14 days we performed clinical examination and determined the dynamics of animal body weight. The mean lethal dose of MSW was determined by probit analysis of lethality curves (Prozorovskiy, 1961). f it was impossible to calculate LD_{50}, the injected amount of the preparation, which did not cause animal death, was fixed.

The resorptive-toxic effect of MSW was studied on 6 white *Wistar* rats weighing 250 g (Kundiev, 1964). The criterion was the presence or absence of lethal cases, time and severity of intoxication manifestations. The preparation in native form was carefully applied to the clipped skin areas of rats at a rate of 8,000 mg kg^{-1}.

Statistical Analysis

MS Excel and the software Statistics, version 7.0, were used for the data analysis. The research results of acute oral toxicity of MSW, given as the mean ± standard error (*SE*). Significance was tested by applying the *Student t-test*. Values of less than 0.05 ($p < 0.05$) were considered significant.

RESULTS AND DISCUSSION

At the first stage of the research, the impact of MSW on soil chemical properties was determined. The main indicators of soil system stability were studied in the scientific chemical-analytical laboratory of Agroecological Monitoring of the Poltava State Agrarian University conducted for 5 years (2017–2021), in particular, the reaction of the soil solution, the content of nitrates, chlorides, mobile sulfur, heavy metals, and petroleum products (Table 1).This research is due to the fact that MSW, in addition to a variety of chemical elements, also contains heavy metals and residual amounts of petroleum products. The plots without MSW and plots which used the full mineral fertilizer $N_{50}P_{50}K_{50}$ were taken as control.

Table 1. Changes in soil chemical parameters when using MSW as the main fertilizer (average for 2017–2021)

Variants of the experiment	pH	Anions, mg kg^{-1}			Petroleum products, mg kg^{-1}	Heavy metals, mg kg^{-1}				Minera-lization, %
		NO_3^-	Cl^-	SO_4^{2-}		Hg	Cu	Pb	Zn	
1. Control	7.6	9.8	131	42.0	330	0.091	0.6	2	28	0.23
2. MSW, 300 L ha^{-1}	6.8	9.8	131	42.0	175	-	0.7	3	17	0.28
3. MSW, 600 L ha^{-1}	6.8	4.9	93	10.2	195	0.065	0.7	3	22	0.24
4. MSW, 900 L ha^{-1}	6.4	8.7	149	40.2	200	0.065	1.0	4	22	0.3
5. MSW, 1,200 L ha^{-1}	6.4	8.7	149	42.8	200	0.052	0.7	4	14	0.21
6. MSW, 2,400 L ha^{-1}	6.2	8.7	149	58.6	200	0.052	0.7	6	16	0.24
7. MSW, 4,800 L ha^{-1}	**5.2**	19.5	224	64.6	320	0.046	0.8	7	24	0.21
8. $N_{50}P_{50}K_{50}$	6.4	30.5	149	34.4	340	0.090	0.8	6	23	0.24

It was found that significant soil acidification occurs only when a dose of MSW higher than 2,400 L ha^{-1} is used. Thus, at the maximum dose of MSW 4,800 L ha^{-1}, pH of the soil solution was 5.2. It should be noted that the use of MSW in the soil solution does not increase the content of nitrates, but rather reduces them, although they are part of its composition. Also the use of MSW in doses of 300–2,400 L ha^{-1} does not contribute to the accumulation of petroleum products and heavy metals in the soil and there is no salinization of the soil. The previous studies (Pysarenko et al., 2021) found that the use of MSW as a herbicide is expedient only in the fields of cereal crops. Therefore, at the second stage, we studied the phytotoxicity of MSW to weeds on winter wheat crops (Table 2).

Table 2. Phytotoxicity of MSW to weeds on winter wheat crops (average for 2017–2021)

Plant	MSW concentration, %			Herbicide Desormone 600 (2.5 kg ha^{-1})
	100	75	50	
	Damage of leaf surface, %			
Caseweed	90.8	89.8	53.7	97.8
Field pennycress	88.9	83.2	38.6	98.0
Winter cress	94.2	86.9	40.4	98.5
Canada thistle	71.5	55.7	30.5	96.9

It was found that after treatment of winter wheat crops in the phase of spring tillering - the beginning of stem elongation, when wheat plants are not sensitive to MSW (Pysarenko et al., 2021), the damage to the leaf surface of weeds is significant. The highest loss of leaf surface was recorded after MSW treatment with a concentration of 100–75%. Burns of the leaf surface of the mentioned weed plants resulted in their death.

Table 3. Weediness of winter wheat crops after MSW treatment (average for 2017–2021)

Variants of the experiment	Biomass of weeds, g		Decrease of dry biomass, %
	Wet weight, g	Air-dry weight, g	
Control (without treatment)	49.2	8.3	-
MSW, 100% concentration	9.6	1.1	85.5
MSW, 75% concentration	11.9	1.9	84.1
MSW, 50% concentration	13.2	2.1	84.1

The data in Table 3 show that the number of weeds is higher on the control than after treatment with different concentrations of MSW. Weed biomass reduction (85.5%) was the largest when MSW concentration of 100% was used. Reducing the number of weeds on the crops leads to increased competitiveness of cultivated plants and improved harvesting conditions. Reduction of weeds on the crops was followed by the increase of winter wheat yields (Table 3).

At the third stage, we studied the effectiveness of MSW on winter wheat crops as a herbicide for 5 years (Table 4).

Table 4. Effects of treatment with different concentrations of MSW on productivity of winter wheat, t ha^{-1}

Variants of the experiment	2017	2018	2019	2020	2021	Average
1. Control (without treatment)	4.11	3.55	2.34	3.49	3.54	3.40
2. Herbicide Desormone, 2.5 kg ha^{-1}	4.74	4.51	3.22	4.42	4.56	4.29
3. MSW, 100% concentration	4,76	4.55	3.29	4.56	4.48	4.33
4. MSW, 75% concentration	4.69	4.49	3.15	4.42	4.27	4.20
5 MSW, 50% concentration	4.10	4.15	2.74	4.29	3.78	3.81

It was established that the average yield of winter wheat over five years was: on control - 3.40 t ha^{-1}, after treatment with herbicide Desormone (2.5 kg ha^{-1}) - 4.29 t ha^{-1}, which is 0.89 t ha^{-1} more than on the control. Yield after treatment with MSW (100% concentration) averaged 4.33 t ha^{-1}, which is 0.93 t ha^{-1} more than control. Yield after MSW treatment (75% concentration) averaged 4.20 t ha^{-1}, which is 0.80 t ha^{-1} more than control. Therefore, the application of 100% and 75% concentration of MSW as a herbicide on winter wheat crops increased the yield by 21.5% and 19.1%, respectively, compared with control.

In order to use any solutions as agrochemicals, it is necessary to carry out their assessment for the environment, warm-blooded animals and bees (Decree The Cabinet of Ministers of Ukraine, 1996). In accordance with World health organization (Executive Committee, 55th session, 1974), it is necessary to carry out their toxicity assessment by LD_{50} in advance. Thus, at the fourth stage, toxicological assessment of MSW was performed according to the following parameters: acute oral toxicity (low-hazard compounds more than 2,000 mg kg^{-1}), resorptive - toxic effect of MSW (low-hazard compounds more than 4,000 mg kg^{-1} (Executive Committee, 55th session, 1974).

Study of acute oral toxicity of MSW. The body weight dynamics of the experimental rats did not differ from the control ($p > 0.05$) (Table 4). It was found that the LD_{50} of MSW for rats in case of oral administration was more than 21,300 mg kg^{-1}.

During the first 1–2 hours after administration of the preparation, the mice showed symptoms of intoxication in the form of immobility, depression, rapid breathing, and movement coordination disorders. The general condition of the survived mice was satisfactory. On day 7, a 10% decrease in body weight gain was observed ($p < 0.05$) (Table 5). It was found that the LD_{50} of MSW for mice when administered orally exceeds 31,000 mg kg^{-1}. According to the parameters of acute oral toxicity for rats and mice, MSW belongs to low-toxic substances according to Executive Committee, 55th session, 1974 (for acute oral toxicity, substances over 2,000 mg kg^{-1} are low-hazard). Variability in species and sex sensitivity to the preparation is insignificant.

Table 5. The research results of acute oral toxicity of MSW

Group of animals	Research period, days		
	0	7	14
	The average weight of rats with acute oral effect of MSW		
Control	229.2 ± 6.2	238.3 ± 6.2	246.7 ± 5.3
Experimental (21,300 mg kg^{-1})	235.0 ± 2.6	240.8 ± 2.6*	250.0 ± 4.4
	Dynamics of body weight of mice under acute oral effect of MSW		
Control	22.6 ± 0.31	26.9 ± 0.92	24.8 ± 0.22
24,000 mg kg^{-1}	21.7 ± 0.25	26.0 ± 0.56*	23.7 ± 0.57
34,000 mg kg^{-1}	22.1 ± 1.30	26.4 ± 0.22*	24.1 ± 0.63
36,000 mg kg^{-1}	21.9 ± 0.54	26.0 ± 0.32*	23.9 ± 1.02

* $p < 0.05$ comparing with control.

The study of the resorptive-toxic effect of MSW. The symptoms of intoxication and death of animals during application of the preparation and throughout the 14-day period were not noted. General condition of the animals and their behavior did not differ from that of the control animals. There was no irritation on the site of MSW application. Thus, MSW had no resorptive-toxic impact on the body of animals when it was put on the skin. The LD_{50} of MSW for rats when applied to the skin is more than 8,000 mg kg^{-1} body weight. According to Executive Committee, 55^{th} session, 1974 (under the resorptive-toxic effect low-hazard substances make more than 4,000 mg kg^{-1}). Therefore, it was determined that MSW belongs to hazard class IV - low-hazard compounds.

CONCLUSIONS

The safety of MSW for the environment in the recommended doses was established as a result of the research. Assessment of MSW impact on soil chemical properties showed that significant soil acidification occurs only when MSW doses higher than 2,400 L ha^{-1} are used. The content of nitrates and petroleum products did not increase and there was no soil salinization when MSW was used in doses of 300–1,200 L ha^{-1}.

The study of MSW phytotoxicity to weeds found that the highest loss of leaf surface was observed after treatment with MSW of 100–75% concentration. The usage of 100% concentration of MSW in a dose of 350 L ha^{-1} resulted in the largest reduction of weed biomass (85.5%). Consequently, winter wheat yield increased by 21.5% when using 100% MSW and by 19.1% when using 75% MSW, while the weediness of crops decreased. As a result of the toxicological assessment of MSW it was found that it belongs to low-toxic compounds. When administered orally, the LD_{50} of white rats is more than 21,000 mg kg^{-1}, of mice - 31,000 mg kg^{-1}; when applied to the skin of rats - more than 8,000 mg kg^{-1}. The value of LD_{50} for female rats is more than 5,000 mg kg^{-1}. The results of the ecotoxicological research of MSW allow us to assert that its application is safe in agriculture, in particular, as an environmentally friendly organomineral fertilizer and herbicide.

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