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Ecotoxicological Risk of Alkaline Sodic Waste on Plant Development

Abstract: The influence of mixtures of soils and Solvay sediments on plant development was investigated under laboratory conditions to explore the possibility of the revegetation of abandoned post-industrial areas. The sodic alkaline wastes used as a substrate in the research come from settling tanks of the former KZS Solvay Works in Cracow. Such sediments are a mixture of different chemical compounds, characterized by high values of pH and salinity that can constitute strong limiting conditions for future reclamation processes of the area. White clover (*Trifolium repens* L.), was used in this research as a plant indicator representative of common and sensitive species to the toxic effects of soil high alkalinity and salinity. This work examined the effect of soda factory waste pollution, mixed with three types of soils in four variants of soil: waste ratio, on germination rate and root length development as response variables. The results, analyzed statistically, confirm an inhibiting effect of soda wastes on *Trifolium repens* L. germination with the significant inhibition of early phase of radicle growth and their elongation. Experiments were conducted on Petri dishes and were based on the Zucconi test. Ecotoxicological risk was evaluated by the percentage of relative seed germination (RSG), relative radicle growth (RRG) and calculated index of germination (GI).

Keywords: Solvay, alkaline sodic wastes, seed germination experiments

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1. Introduction

Very unfavorable chemical properties of the waste of soda production cause settling tanks to be specific and problematic sites to conduct environmental reclamation [1–3]. In the classification of post-industrial areas proposed by Krzaklewski [4], based on the observation of land cover as a result of spontaneous succession, post-sodic settlers are described as objects which are very difficult for biological reclamation with no spontaneous overgrowth for at least 10 years. Such waste is characterized by extremely high pH values and a significant degree of salinity [3, 5, 6].

The topsoil layer was applied to the crown of the sedimentation tanks in the study area during an attempt at a reclamation process. Overburden material was characterized by differences in thickness and diversity in texture. Previous studies [3, 7] have shown that the used topsoil was affected by contact with waste inducing changes in its physical and chemical properties. The soil layer was often displaced and mixed with the covered waste by the presence of animals that spend most of their lives underground or under the topsoil overburden. It causes mixing waste with soil in varying proportions.

The waste degree of phytotoxicity depends on the cumulative impact of different parameters, this aspect being particularly complex in waste which are mixtures of different compounds (in this case: CaCO_3 , CaO , SiO_2 , R_2O_3 , MgO , CaSO_4 , Na_2O , CaCl_2 , Na_2CO_3 , NaCl and others) [8] which together may lead to complex interactions which are different from the additive effects of individual components [9]. It is difficult to unequivocally assess the impact of substrate limitation of plants growth only on the basis of chemical analysis. Biological test methods for estimating the risk of substrate phytotoxicity allow to describe more reliable conclusions [9, 10]. Zucconi based tests [11] are frequently used for evaluating seed germination and plant growth on a selected substrate by counting the percentage of relative seed germination (RSG), relative radicle growth (RRG) and calculated index of germination (GI).

2. Materials and Methodology

Ecotoxicity risk evaluation was performed using the Zucconi methodology [10]. White clover (*Trifolium repens* L.) seeds were placed on Petri dishes and germination was initiated by wetting the seeds with four soil : waste water extracts from the saturated paste (in weight ratio soil to waste 4 : 0, 3 : 1, 2 : 2 and 1 : 3) testing three soils texture types: loamy sand (LS), sandy loam (SL) and loam (L). Soil and waste particles were removed by centrifugation and later by filtration, obtaining the solution for wetting the seeds. Eight seeds were placed in each Petri dishes with germination paper and 1 ml of extract was dropped. Petri dishes were closed with parafilm tape to minimize possible evaporation. The experiment was performed with 10 replicas

of each treatment. The seed germination in soil water saturated paste extracts (soil : waste 4 : 0) were treated as control probes. All extracts were checked by pH and EC measurements (Tab. 1). Plant incubation was performed under darkness conditions at a temperature of 28°C. After 48 hours of incubation, the number of seeds germinated and the root length of the plants was measured.

Table 1. The pH and EC values of the filtrate extract for all experimental variants

Ratio soil : waste (w : w)	Loamy sand (LS) 1		Sandy loam (SL) 29		Loam (L) 24	
	pH	EC [$\mu\text{S}/\text{cm}$]	pH	EC [$\mu\text{S}/\text{cm}$]	pH	EC [$\mu\text{S}/\text{cm}$]
4 : 0	7.16	55.3	6.76	59.3	7.19	78.9
3 : 1	10.09	372	9.65	364	9.68	368
2 : 2	10.74	571	10.50	620	10.45	570
1 : 3	10.80	829	10.83	796	10.80	836

White clover (*Trifolium repens* L.) was used in this experiment as a plant which is sensitive to a saline and highly alkaline environment [12, 13] of the type which occurs in Solvay waste. White clover is also a common component of grass mixes for different reclamation purposes. This widespread grassland species is quick to germinate. In natural conditions, seed germination occurs in the soil within 24 hours at a temperature of around 18–20°C [14]. The wide range of temperatures and light conditions that stimulates germination [15] and the high germination rate of seeds reported for around minimum of 73% [13] allow the easy use of this species in germination research.

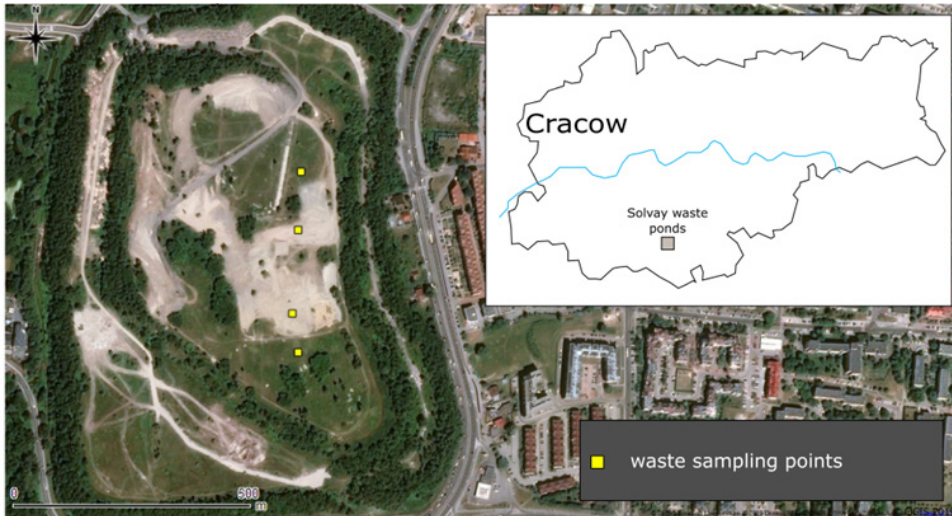


Fig. 1. Location of waste sampling point

Waste probes came from the Solvay waste ponds from the former KZS Solvay (Krakow Soda Works) factory in Krakow (complex of sediment ponds no. 2). It was taken as a composite sample from four sampling points (Fig. 1). Waste was characterized by very high pH (12.0) and EC 3.0 mS/cm. The particle size distribution of the waste was measured by means of a particle size analyzer IPS-U (Kamika Instruments) because of their physical and chemical properties that do not allow their measurement by means of other laboratory methods. Waste "texture" was described by the USDA soil classification as a "Silt".

Soil material was taken from non-urbanized areas. Soil samples present three different textures: sandy loam (SL), loam (L) and loamy sand (LS). The pH measured in H₂O (1/5 soil water extracts w/v) was 7.4, 7.34 and 7.56 respectively. Electrical conductivity (in 1/5 soil water extracts w/v) was 90 (SL), 225 (L) and 156 (LS) expressed in microsiemens per centimeter [μ S/cm].

3. Results

Data sets were divided into three groups according to the soil used in experiment (SL, L, LS) and four variants depending on soil : waste ratio (4 : 0, 3 : 1, 2 : 2 and 1 : 3). The following abbreviated terms were used in this paper to clarify precisely each data set among each soil type and soil : waste ratio: LS_{4:0'} LS_{3:1'} LS_{2:2'} LS_{1:3} SL_{4:0'} SL_{3:1'} SL_{2:2'} SL_{1:3} L_{4:0'} L_{3:1'} L_{2:2'} L_{1:3'} where letters mean soil texture and subscripts behind the letters represent the weight ratio between the quantity of soil and waste.

After the end of the germination, the number of seeds germinated in the control and in each treatment were annotated for each of the ten replicas. Radicle longitudes were also measured, and results were analyzed statistically and the indicators of the germination process computed.

Average values of radicle longitude decreased with the decrease in the ratio of soil : waste in all soil types. The highest difference was observed between LS_{4:0} and LS_{1:3} (from 15.7 to 6.13 mm) and for SL_{4:0} and SL_{1:3} (from 16.9 to 5.6 mm) (Tab. 2). For those in variant 1 : 3 25% of observation are seeds that did not germinate. The 75% of measured radical longitude in L (loam) reach values up to 26 mm (L_{4:0}), 21 mm (L_{3:1}), 19 mm (L_{2:2}) and 18.75 mm (L_{1:3}) and are higher than in LS and SL cases.

The existence of anomalous observations in data sets of radicle longitude was checked by means of the interpretation of box-plots. Outlier detection was made using box-and-whisker plots: the data observations which are below Q1 - 1.5 IQR and the data observations that are over Q3 + 1.5 IQR, are considered outliers, (IQR is the interquartile range, Q1 is 25 percentile and Q3 is 75 percentile). Extreme values outside the outer fences (Q1 - 3 IQR and Q3 + 3 IQR) were not detected in any data sets. To statistical analyses all outliers' values (when occurred) were replaced with the next lowest non-outlier value in each data set.

Table 2. Basic statistics of radicle longitude of plants growing in different variants

Radicle longitude [mm] in different variants	Min.	Max.	Mean	Median	Variance	Standard dev.	25 th percentile	75 th percentile
LS _{4:0}	0	27	15.70	17.5	49.9721	6.8536	12.25	20.75
LS _{3:1}	0	32	12.79	13.0	59.4099	7.7078	7.50	19.00
LS _{2:2}	0	29	10.75	11.5	44.0886	6.6399	6.00	16.00
LS _{1:3}	0	22	6.13	3.0	46.9968	6.8554	0.00	12.00
L _{4:0}	10	35	20.91	21.0	40.7138	6.3807	18.00	26.00
L _{3:1}	0	28	13.21	14.0	86.0429	9.2759	3.50	21.00
L _{2:2}	0	32	11.45	11.0	90.5038	9.5133	0.00	19.00
L _{1:3}	0	30	12.28	13.0	66.3846	8.1476	5.00	18.75
SL _{4:0}	3	32	16.90	19.0	58.5215	7.6499	13.25	21.00
SL _{3:1}	0	31	12.29	15.0	90.6631	9.5217	0.00	20.00
SL _{2:2}	0	28	9.72	11.5	78.2525	8.8460	0.00	16.00
SL _{1:3}	0	23	5.60	0.0	60.2936	7.7649	0.00	13.75

The ANOVA Kruskal–Wallis test was used to compare radicle length within the soil : waste ratio variants as well as within each type of soil (Tab. 3). The test does not require assumptions that distributions of variables have to be close to the normal distribution. Other requirements for the Kruskal–Wallis, as the quantitative scale of dependent variables and independency of observations in the analyzed groups, were fulfilled. Test results (difference identification as statistical significance) provided the basis for detailed analysis.

Table 3. Kruskal–Wallis test results (radicle longitude) in two type of data set divisions

Within the type of soil used for the experiment	H-value	<i>p</i>	Within the soil : waste ratio used for the experiment	H-value	<i>p</i>
LS	63.28	$8.42 \cdot 10^{-14}$	4 : 0	23.35	$8.14 \cdot 10^{-6}$
L	54.10	$9.13 \cdot 10^{-12}$	3 : 1	0.47	0.7879
SL	65.88	$1.16 \cdot 10^{-14}$	2 : 2	1.271	0.5226
			1 : 3	31.64	$4.68 \cdot 10^{-8}$

Results of Kruskal–Wallis test are placed in Table 3 in two data sets divisions:

- 1) “Soil texture division”: The test probability in all examined cases was lower than the assumed level of significance $\alpha = 0.05$ and shows statistically significant differences within soil texture groups.
- 2) “Soil : waste ratio division”: The test probability was lower than the set level of significance α in the cases of variant (4 : 0) and (1 : 3), within those variants statistically significant differences were showed.

More detailed analysis (within detected differences) was made using the *U*-Mann–Whitney nonparametric test.

In the “soil texture division”, statistical differences between each bordering ratio variants were analyzed in subsequential pairs and are shown in Table 4.

Table 4. *U*-Mann–Whitney test statistics in comparing pairs of variants

Compared pair of variants	<i>U</i> -value	<i>z</i> -value	<i>p</i>
LS			
LS _{4:0} vs LS _{3:1}	2428.0	-2.6383	0.0077
LS _{3:1} vs LS _{2:2}	2645.5	-1.8961	0.0552
LS _{2:2} vs LS _{1:3}	2015.5	-4.0985	0.0001
L			
L _{4:0} vs L _{3:1}	1708.0	-5.1000	0.0001
L _{3:1} vs L _{2:2}	2820.5	-1.3042	0.1914
L _{2:2} vs L _{1:3}	2995.5	-0.7006	0.4749
SL			
SL _{4:0} vs SL _{3:1}	2254.5	-3.2339	0.0015
SL _{3:1} vs SL _{2:2}	2648.0	-1.9185	0.0529
SL _{2:2} vs SL _{1:3}	2417.0	-2.8271	0.0043

In soil : waste pairs LS_{4:0} vs LS_{3:1}, LS_{2:2} vs LS_{1:3}, L_{4:0} vs L_{3:1}, SL_{4:0} vs SL_{3:1}, SL_{2:2} vs SL_{1:3} *U*-Mann–Whitney test confirming statistically significant differences between variants (Tab. 4). In other cases, the differences were not significant.

The *U*-Mann–Whitney test was also used to check differences between soils type used for the experiment within the 1 : 3 ratio (Tab. 5). Data only shows significant differences between loam and other soils. In comparison of sandy loam and loamy sand, the differences were not statistically significant.

Table 5. *U*-Mann–Whitney test statistics in comparing soils using to experiment in variant 1 : 3

Pairs of soil within 1 : 3 ratio	<i>U</i>	<i>z</i>	<i>p</i>
LS vs L	1810	-4.74183	$1.483 \cdot 10^{-6}$
L vs SL	1754	4.93294	$4.118 \cdot 10^{-6}$
LS i SL	2967	0.79343	0.39361

Germinated seeds were also counted on Petri dishes and summarized. Table 6 and Figure 2 show changes in germination as well as the diversity of results.

Table 6. The RSG, RRG and GI values

	Loamy sand (LS) : waste			Sandy loam (SL) : waste			Loam (L) : waste		
	3 : 1	2 : 2	1 : 3	3 : 1	2 : 2	1 : 3	3 : 1	2 : 2	1 : 3
RSG [%]	90.54	91.89	59.46	81.43	71.43	48.57	87.50	79.17	93.06
RRG [%]	89.96	74.51	65.61	91.32	82.39	69.77	76.84	73.60	67.20
GI [%]	81.45	68.47	39.01	74.36	58.85	33.89	67.24	58.27	62.54

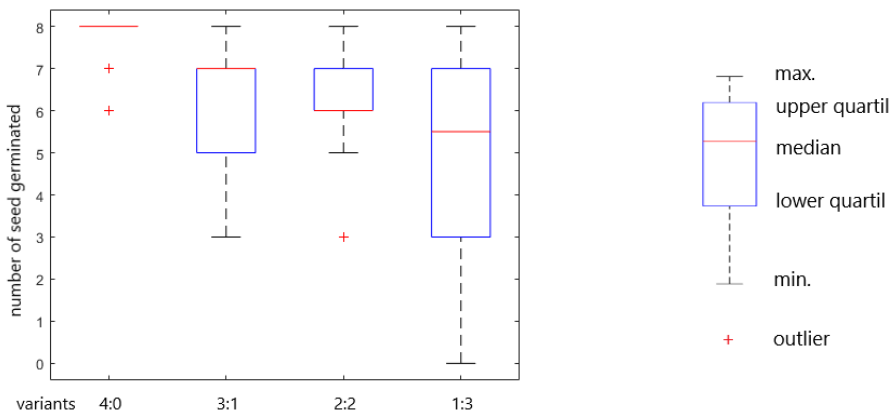


Fig. 2. Distribution of selected statistics of the number of germinated seeds in different variants soil : waste

The percentage of relative seed germination (RSG), relative radicle growth (RRG) and index of germination (GI) were calculated according to formulas used widely by other researchers [11, 16, 17]:

$$RSG = \frac{\text{number of germinated seeds in sample}}{\text{number of germinated seeds in control sample}} \times 100 [\%] \tag{1}$$

$$RRG = \frac{\text{total radicle length of seeds in samples}}{\text{total radicle length of seeds in control sample}} \times 100 [\%] \tag{2}$$

$$GI = \frac{RSG \times RRG}{100} [\%] \tag{3}$$

The germination index (3) is calculated from both the radicle length percentage and germination percentage of the seeds in the probes compared to control probe.

A GI value of more than 80% usually means no phytotoxicity problems [15]. There is only one sample, the LS_{3,1}, which exceeds this level. The phytotoxicity state can be noticed in variants with a higher amount of waste (SL_{1,3} LS_{1,3}). In the rest of the variants, slight phytotoxicity occurred. Differences in root length (in different soil : waste variants) in relation to control samples, expressed by the RRG index, (percentage of relative root length) decreases from 86% (average for options 3 : 1) to 67.5% (average for options 1 : 3). Relative seed germination was low for LS and SL variants 1 : 3. For loam soil, the RSG values were high and stable, although RRG is a more sensitive indicator than RSG for toxicity [16].

4. Conclusions

It was expected that with the increase waste weight in soil : waste ratio, there would be an increase in the pH and PEW values of the extract prepared for the experiment. Seed germination sensitivity to environmental pollution allows the evaluation of phytotoxicity in a short-time experiment.

The experiment led to the following conclusions:

- The average root length decreases with a decrease in the ratio soil : waste.
- The percentage of seeds that have not germinated in all types of soils increase respectively from 10% (for variant 4 : 0) to 22%, 27% and 39% (in variant 3 : 1).
- Statistical tests confirm that the presence of waste in combination with soil has a significant effect on medium phytotoxicity. It shows statistically significant differences within soil texture groups and in variants of soil : waste ratio 4 : 0 and 1 : 3.
- Changes in root length (in different soil : waste variants) in relation to control samples expressed by the RRG index (percentage of relative root length) decreases from 86% (average for options 3 : 1) to 67.5% (average for options 1 : 3). The RRG index changes confirms the other analysis of the results, indicating statistically significant differences between the variants (from 3 : 1 to 1 : 3) in the measured root length for each of the tested soils.
- The RSG value (percentage of relative seed germination) decreases from 86.5% (average for variants 3 : 1) to 67% (average for variants 1:3).
- Increasing the amount of waste in subsequent options resulted in significant decreases in the germination rate of GI.
- Only the GI of the LS_{3,1} variant indicated the non-phytotoxic state, while others (LS_{2,2'} L_{3,1'} L_{2,2} L_{1,3'} SL_{3,1'} SL_{2,2'}) are a slightly phytotoxic or phytotoxic (SL_{1,3'} LS_{1,3}) medium to grow in.
- Statistical analyses have shown that the differences between loam, sandy loam and loamy sand are statistically significant. In the case of loam, changes in the concentration of waste do not have a significant impact on the phytotoxicity of the substrate for plant growth and seems to provide stable conditions for the germination and growth of root parts of the plants.

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Ocena ryzyka ekotoksykologicznego związanego z wpływem alkalicznych odpadów posodowych na rozwój roślin

Streszczenie: Celem pracy było zbadanie, w warunkach laboratoryjnych, wpływu odpadów przemysłu sodowego na rozwój roślin. Odpady wykorzystywane w badaniach pochodzą z osadników byłych Zakładów Sodowych Solvay w Krakowie. Osady takie są mieszaniną różnych związków charakteryzujących się wysokim poziomem pH i zasolenia, co może stanowić silną barierę dla przyszłych procesów rekultywacji jako antropogeniczny czynnik stresogenny dla rozwoju roślin. W badaniach jako wskaźnik roślinny wykorzystano koniczynę białą (*Trifolium repens L.*), gatunek powszechnie występujący i wrażliwy na toksyczne skutki wysokiego zasolenia. W pracy zbadano wpływ odpadów sodowych na kiełkowanie roślin w trzech typach gleb oraz w czterech wariantach stosunku gleba : odpady. Przenalizowane statystycznie wyniki potwierdzają hamujący wpływ odpadów na kiełkowanie *Trifolium repens L.* z istotnym zahamowaniem wczesnej fazy wzrostu korzeni i ich długości. Eksperymenty przeprowadzono na szalkach Petriego, posługując się testem Zucconiego. Ryzyko ekotoksykologiczne oceniono na podstawie procentu względnego kiełkowania nasion (RSG), procentu względnego wzrostu korzeni (RRG) oraz obliczonego wskaźnika kiełkowania (GI).

Słowa

kluczowe: Solvay, odpady posodowe, kiełkowanie roślin