Polyoxometalates of Keggin Type with Mixed Addenda
Used as Fertilizers for *Triticale* Seeds

ANDA IOANA GRATIELA PETREHELE*, DAN RUSU†, MONICA ANGELA SIPOS*, ALEXANDRINA FODOR*, MARIANA RUSU†

*University of Oradea, Faculty of Sciences, 1 Universitii Str., 410087, Oradea, Romania
†Medicine and Pharmacy "Iuliu Hateganu" University, Faculty of Pharmacy, 13 Emil Isac Str., 400023, Cluj-Napoca, Romania
‡Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, 1 Arany Janos Str., 40028, Cluj-Napoca, Romania

In this paper, we propose to follow the influence of the monolacunary Keggin polyoxoanion $K_x[MPVW_{10}O_{39}(H_2O)]$ and its complexes with transition metals: $K_x[MPVW_{10}O_{39}(H_2O)]$ (x = 6 for M = Mn(II), Cu(II), Co(II), Ni(II) and x = 5 for M = Fe(III)) in the germination of the *Triticale* seeds. The *Triticale* seeds were treated with solutions of each species in concentrations from 0.1 to 100 ppm, and the measured parameters have been reported to seeds treated with distilled water as control. Parameters followed along our studies were: the efficiency of the germination, the length of the radicles and seminal roots, the length of the coleoptile and the first foliage leaf, the biomass yield. Mn(II), Co(II), Ni(II) and Fe(III) compounds stimulated growth of radicles and seminal roots, especially at lower concentrations. The Cu(II) complex inhibited generally the growth of all anatomical parameters, but the biomass increase was stimulated especially in treatment with 1.0 ppm solution. The monolacunary polyoxoanion, $K_x[MPVW_{10}O_{39}(H_2O)]$, was the best fertilizer of the studied species due to its ability to act as a polydentat ligand for transition metal cations. Its action on germination and growth seedlings was better than for its coordinated compounds with cations. Concentrations of transition metal cations were measured for seedlings by the germination of *Triticale* seeds and it was seen that these were accumulated in plants. The cations accumulation in plants increased in the same time with the concentration of $K_x[MPVW_{10}O_{39}(H_2O)]$ solutions.

Keywords: Keggin, polyoxometalates, mixed ligands, germination seeds, *Triticale*

The Keggin polyoxometalates have general formula $[X_n^{m+}M_{12}O_{40}]^{2k-}$ and they are polycondensation products between a $XO_4$ central unit ($X = P (V), As (V), Si (IV), B (III), etc.$) and twelve $MO_6$ octahedral units (M = W (VI), Mo (VI), V (V), Nb (V)), bound together by common corners and edges. The Keggin monolacunary species $[X_n^{m+}M_{12}O_{40}]^{2k-}$ are obtained by removing the $M=O$ unit and acts as a polydentat ligand for metal transition ions. In these compounds, transition metal ions are surrounded octahedral generally. The monolacunary Keggin $[X_n^{m+}M_{12}O_{40}]^{2k-}$ can bind to a transitional metal ion by five oxygen atoms and the sixth coordination position is provided by an external ligand, L, where (L=H$_2$O, OH$, O^2$, O$_2$-, etc.) [1, 2].

A feature of Keggin polyoxometalates is multiple equilibrium that can occur between different polyoxometalate structures (complete and lacunary) and simple ions of the elements. The direction of the chemical reaction equilibrium depends very much on external factors (pH, temperature, reducing agents, counter ion, solvent) [1, 2].

Polyoxometalates have complex structures with more various elements and special redox properties, so that they have many applications, especially in analytical and clinical chemistry, in homogeneous and heterogeneous catalysis, in medicine (antiviral, antibacterial, anti-HIV activity), electrochemistry, biochemistry (implication in enzymatic activity) and in development of more other research fields [3-5].

It is known that the plants need for their growing a large number of nutritive elements. Some are absolutely essential for plants life, and experts have grouped them in: macro elements, necessary for plant in quantity more than 0.01% in dry weight (C, O, H, N, P, K, Ca, Mg, Na and Cl); trace elements necessary in smaller quantity (0.01 – 0.00001%): Fe, Mn, Cu, Zn, B, Mo, Co, V; and ultramicro elements, necessary in very small quantity (under 0.00001% in dry weight) [6]. The macro elements are involved in anatomical structure of the plants and in their important physiologic processes, so that they have a very important place in plants development and evolution. The macro elements increase the resistance of the plants to aggressive environmental factors and pests and they contribute to biomass accumulation [7-11].

Essential trace elements discussed in this paper (V, Co, Cu, Ni, Mn and Fe) are involved mainly in redox properties of enzymes, leading to increased plant biomass. These trace elements in high concentration have toxic and stressed actions on plant evolution. The metabolic and growth processes of the plants can be slowed down or blocked at great concentration of trace elements. From literature data the order of toxic action of M cations on the plants growth are: Cu M (II)> Co (II) > Ni (II)> Mn (II)> Fe (III) [12-17]. Some plants have developed strategies to adapt to high concentrations of trace elements [18-22].

Studies on plants, showed that vanadium ions are localized mainly in the leaves, ions of copper, cobalt and iron are fixed in the roots, and manganese and nickel are distributed equally throughout the plant [22,23]. Tungsten cations should behave similar to those of molybdenum, so they should localize mainly in roots [23].

In this paper we have proposed to follow the action of a series of Keggin polyoxometalates on *Triticale* seed germination. The using of polyoxometalates with mixed addenda as fertilizers opened a new exploration way of their special properties. The influence on seeds germination was studied under laboratory conditions, in the dark at temperature and humidity unmodified. The experiments were intended the degree of germination, the evolution of the anatomical components of seedlings on
the sixth day after germination (the length of radicle, seminal (adventitious) roots, coleopille and first foliage leaf) and the biomass yield. The metallic cations concentrations in seedlings were measured and all results were statistically processed.

Experimental part

Seeds were selected from species Triticale, which is a hybrid of wheat and rye. Triticale seeds used had germination of 94%, humidity of 14.4%. The seeds were washed with distilled water, dried and sorted before use.

The Keggin series taken in work to treat seeds of Triticale consisted of monolacunary ligand, $K_8[PVW_{10}O_{39}]·15H_2O$ (L), and its complexes with transition metal ions: $K_8[CoPVW_{10}O_{39}]·14H_2O$ (CoL), $K_8[CrPVW_{10}O_{39}]·21H_2O$ (CrL), $K_8[NiPVW_{10}O_{39}]·19H_2O$ (NiL), $K_8[MnPVW_{10}O_{39}]·14H_2O$ (MnL), $K_6[FePVW_{10}O_{39}]·6H_2O$ (FeL).

Polyoxometalates were prepared by synthetic methods mentioned in the literature [24-27]. Synthesis was carried in two steps: first step was the synthesis of monolacunary polyoxometalate, $K_8[PVW_{10}O_{39}]$, by mixing $NaH_2PO_4·H_2O$, $NaWO_2·2H_2O$ and $NaV_2O_5·4H_2O$ in the presence of $HCl$, followed by addition of $KCl$. In the second step, monolacunary polyoxometalate, $K_8[PVW_{10}O_{39}]$, was combined in equimolecular ratio with a metalic cation salt for obtaining by $K_x[MPVW_{10}O_{39}(H_2O)]$ coordination complex. All chemicals used were of high analytical purity and generic reactions (1) and (2) revealed how these compounds were synthesized:

$$NaH_2PO_4 + 10NaWO_2·2H_2O + NaV_2O_5·4H_2O + 14HCl + 8KCl \rightarrow$$
$$K_8[PiV_2O_9]·22NaCl + 8H_2O$$

Thermal analyses were effected with a derivatograph model Thermal analyses were effected with a derivatograph type V arrian ASA 220. An IR spectrophotometer type Biorad FTS 60A (with frequency type Paulik-Erdely OD-103. For the analytical determinations consisted of monolacunary ligand, $K_8[PVW_{10}O_{39}]·15H_2O$ (L). Elemental analysis and TG data (found (calc.)): K 7.50 (7.28); P 0.92 (0.96); V 1.50 (1.58); W 57.00 (58.88); Co 1.95 (2.04); H 0.87 (0.87). FTIR bands ($\nu_{\text{max}}, \text{cm}^{-1}$): 3435, 1624, 1086 m, 1064.5 m, 953 s, 879.5 m, 815 vs, 760 s, 716 sh.

Solutions of each polyoxometalate species with the next concentrations were prepared: 10^{-7} M · L^{-1} (0.1 ppm), 10^{-6} M · L^{-1} (1.0 ppm), 10^{-5} M · L^{-1} (10 ppm), 10^{-4} M · L^{-1} (100 ppm). The control solution was distilled water.

In each germinator, Triticale seeds were placed carefully on a bed of filter paper and they were wetted with 25 mL of one of the prepared solutions (distilled water or solution of polyoxometalates). Each germinator was closed and was put in a dim and isolated room, at 21-23°C. In the fourth day germinated seeds were counted. Seeds were soaked again with 25 mL of the same solution and kept for two days, under the above conditions. In the sixth day, the germinated seed were counted and the anatomic parts of the seedlings were measured. To determine biomass was used a heating and drying Thermo Heraeus oven.

Dried seedlings in each experiment were mineralized and M cations concentration was determined with a spectrophotometer type Varian ASA 220.

TTEST function in Excel 2007 software was used for statistical interpretation of the results. TTEST function shows that it is likely that two sets of values coming from the same two underlying populations to have the same average. If the probability is $p < 0.05$ we conclude that it is unlikely that the difference observed between the averages of sample and control to be by chance (random sampling). In other words, we conclude that two populations studied are indeed different environments [27].

Statistical interpretation of results was achieved by plotting the percentage differences, comparing the change averaged between samples and control (eq. 1). Percentage differences show if there is a significant difference between sample and control.

$$\% \text{ Differences} = \frac{(\bar{x}_{\text{sample}} - \bar{x}_{\text{control}}) \times 100}{\bar{x}_{\text{control}}}$$  

Results and discussions

The number of seeds germinated in the fourth day and sixth day are recorded in table 1. In the tabular data can be seen that the efficiency of germination was more than 75% in almost all experiments. The highest yields were obtained from germinating seed treatment with solutions: 1 ppm Col (96%), 10 ppm MnL (94%) and 0.1 ppm FeL (92%). A decline of germination rate compared to control was obtained in the following situations: 1.0 ppm CuL (76%), 100 ppm CuL (73%) and 1.0 ppm MnL (71%). As shown in table 1 the best germination rates compared with control, for each polyoxoanion were obtained as follows: 1 ppm Col (96%), 10 ppm MnL (94%) and 0.1 ppm FeL (92%).

Table 2 shows the results obtained from statistical processing of data using T TEST. Significant influence on statistical parameters seedlings resulting from germination of Triticale seeds were obtained in all cases where $p < 0.05$. All these results were interpreted using percentage differences from control in graphical representations in figures 1-6.

Table 1 shows the results obtained from statistical processing of data using T TEST. Significant influence on statistical parameters seedlings resulting from germination of Triticale seeds were obtained in all cases where $p < 0.05$. All these results were interpreted using percentage differences from control in graphical representations in figures 1-6.
In figure 1 was showed the influence of L solutions on Triticale seedlings compared with the control. The L solution used, from 0.1 to 100 ppm concentrations, stimulated the growth of anatomical parameters of the seedlings. Also, it can be seen that the growth of radicle and seminal roots was stimulated at seeds treatment with L solutions of the following concentrations: 10 ppm (23% and 18.78%) and 100 ppm (19.8% and 13.94%). The coleoptile growth was significant only for 10 ppm L solution (5.31%), and the growth of the first leaf was higher significantly in treatment with 100 ppm L solution (22.92%). The influence on the biomass growth was significant at 100 ppm L (7.80%). The biomass accumulation was inhibited at 0.1 ppm L.

In figure 2 we can see that the treatment of Triticale seeds with 0.1-100 ppm CuL solutions inhibited the increased of the most seedlings parameters used in study. 0.1 ppm CuL solution inhibited increase of the seminal roots (-15.58%) and the growth of another parameters were not influenced. The 1 ppm CuL solution inhibited significantly the growth of radicle (-13.84%), seminal roots (-21.62%), coleoptile (-10.13%) and first leaf (-14.55%), but the biomass yield (8.24%) were stimulated significantly. 10 ppm solution CuL stimulated significantly radicle growth (10.04%), but inhibited seminal roots growth (-11.90%). 100 ppm CuL solution stimulated radicle growth (-6.24%) and biomass accumulation (5.71%), but increased in length of seminal roots (-16.52%) and the first leaf (-6.54%) was inhibited.

In figure 3, the treatment with 0.1-100 ppm CoL solutions stimulated the growth of radicles and seminal roots. The largest increase of the radicles occurred in 0.1 ppm CoL (21.05%) and of the seminal roots was at increased of the most seedlings parameters used in study. 0.1 ppm CuL solution inhibited increase of the seminal roots (-15.58%) and the growth of another parameters were not influenced. The 1 ppm CuL solution inhibited significantly the growth of radicle (-13.84%), seminal roots (-21.62%), coleoptile (-10.13%) and first leaf (-14.55%), but the biomass yield (8.24%) were stimulated significantly. 10 ppm solution CuL stimulated significantly radicle growth (10.04%), but inhibited seminal roots growth (-11.90%). 100 ppm CuL solution stimulated radicle growth (-6.24%) and biomass accumulation (5.71%), but increased in length of seminal roots (-16.52%) and the first leaf (-6.54%) was inhibited.

In figure 3, the treatment with 0.1-100 ppm CoL solutions stimulated the growth of radicles and seminal roots. The largest increase of the radicles occurred in 0.1 ppm CoL (21.05%), and of the seminal roots was at

**Table 1**

<table>
<thead>
<tr>
<th>Keggin polyoxometalates used</th>
<th>Radicle root (cm)</th>
<th>Seminal roots (cm)</th>
<th>Coleoptile (cm)</th>
<th>First foliage leaf (cm)</th>
<th>Biomass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L 0.1 ppm</td>
<td>0.20</td>
<td>0.04</td>
<td>0.13</td>
<td>0.34</td>
<td>0.19</td>
</tr>
<tr>
<td>1.0 ppm</td>
<td>0.16</td>
<td>0.21</td>
<td>0.21</td>
<td>0.37</td>
<td>0.47</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.01</td>
<td>0.00</td>
<td>0.04</td>
<td>0.21</td>
<td>0.44</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.02</td>
<td>0.01</td>
<td>0.27</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>CuL 0.1 ppm</td>
<td>0.46</td>
<td>0.05</td>
<td>0.27</td>
<td>0.42</td>
<td>0.43</td>
</tr>
<tr>
<td>1.0 ppm</td>
<td>0.10</td>
<td>0.00</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.16</td>
<td>0.02</td>
<td>0.14</td>
<td>0.39</td>
<td>0.47</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.27</td>
<td>0.00</td>
<td>0.24</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>CoL 0.1 ppm</td>
<td>0.02</td>
<td>0.23</td>
<td>0.42</td>
<td>0.27</td>
<td>0.13</td>
</tr>
<tr>
<td>1.0 ppm</td>
<td>0.05</td>
<td>0.00</td>
<td>0.03</td>
<td>0.26</td>
<td>0.17</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.23</td>
<td>0.09</td>
<td>0.04</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.04</td>
<td>0.02</td>
<td>0.12</td>
<td>0.37</td>
<td>0.01</td>
</tr>
<tr>
<td>NiL 0.1 ppm</td>
<td>0.05</td>
<td>0.02</td>
<td>0.31</td>
<td>0.38</td>
<td>0.03</td>
</tr>
<tr>
<td>1.0 ppm</td>
<td>0.35</td>
<td>0.05</td>
<td>0.15</td>
<td>0.42</td>
<td>0.18</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.41</td>
<td>0.46</td>
<td>0.04</td>
<td>0.42</td>
<td>0.25</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.43</td>
<td>0.46</td>
<td>0.39</td>
<td>0.39</td>
<td>0.00</td>
</tr>
<tr>
<td>MnL 0.1 ppm</td>
<td>0.02</td>
<td>0.00</td>
<td>0.09</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>1.0 ppm</td>
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<td>0.22</td>
<td>0.45</td>
<td>0.18</td>
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<td>0.32</td>
<td>0.35</td>
<td>0.46</td>
</tr>
<tr>
<td>100 ppm</td>
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<td>0.45</td>
<td>0.15</td>
<td>0.26</td>
<td>0.22</td>
</tr>
<tr>
<td>FeL 0.1 ppm</td>
<td>0.00</td>
<td>0.04</td>
<td>0.07</td>
<td>0.39</td>
<td>0.11</td>
</tr>
<tr>
<td>1.0 ppm</td>
<td>0.21</td>
<td>0.43</td>
<td>0.28</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.04</td>
<td>0.19</td>
<td>0.07</td>
<td>0.35</td>
<td>0.01</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.22</td>
<td>0.47</td>
<td>0.02</td>
<td>0.32</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Fig. 1. Percentage differences of seedlings parameters after treatment with L solutions (R-radicle, SR-seminal roots, C-coleoptile, FL-first leaf, B-biomass)
treatment with 1.00 ppm CoL solution (22.12%). The coleoptile growth was stimulated by 1.00 to 100 ppm CoL, but the coleoptile increasing was not enough to be significant. Increase of the first leaf was not stimulated significantly for any of 0.1 to 100 ppm CoL solutions, over the treatment with 10 ppm solution led to a significant inhibition (-5.83%). In all treatments with CoL solutions, the biomass concentration decreased significantly compared with control, the greatest inhibition of biomass yield occurred in treatment with 100 ppm CoL solution (-9.78%).

As shown in figure 4, at the treatment of Triticale seed with 0.1-100 ppm NiL solutions, the increasing of radicle was stimulated, especially for the 0.1 ppm NiL solution (7.14%). The growth of seminal roots was stimulated significantly for 0.1 ppm NiL solution (7.13%) and 1 ppm (7.13%) NiL solutions and it was inhibited at 10 and 100 ppm concentrations. The coleoptile increased significantly only at treatment with 10 ppm NiL solution (7.37%) and it was inhibited at 0.1 and 100 ppm NiL solutions. There were no significant differences in growth of the first leaf between control and NiL solutions used to the treatment of Triticale seeds. Biomass yield was inhibited for all four NiL solutions taken in the work further the growth inhibition was significant at 0.1 ppm (-7.08%) and 100 ppm (-9.99%) NiL.

As shown in figure 5, variations of anatomical parameters of Triticale seedlings treated with 0.1 to 100 ppm MnL solutions has been particularly at 0.1 ppm concentration, while the variations observed at 100 ppm concentration were not significant. Significant increases in seedlings after the treatment with 0.1 ppm MnL solution were the following: 19.73% for radicals, 21.01% for seminal roots and 8.98% for first leaf. However, biomass yield was strongly inhibited in the experiment with 0.1 ppm MnL solution (-11.75%).

In figure 6, the radicle growth of seedlings was stimulated in 0.1-100 ppm FeL solutions and the highest increase was at treatment with 0.1 ppm FeL solution (31.58%). The seminal roots growth was stimulated by 11.26% at the treatment in 0.1 ppm FeL solution. The coleoptile growth was inhibited in the Triticale seed treatment in all FeL solutions taken in study, especially in 100 ppm FeL solution (-5.66%). Stimulation/inhibition of first leaf growth was insignificant compared with control at 0.1-100 ppm FeL solutions. The increasing of biomass in seedlings was inhibited in FeL solutions, especially in 10 ppm (-11.81%) and 100 ppm (-11.74%) concentrations.

In table 3 it can be seen that significant variation of M cations concentrations were recorded beginning with 0.1 ppm CoL solution, 1.0 ppm concentration for CoL and NiL solutions, 10 ppm concentration for MnL and FeL solutions. The concentrations values proved that M cations
assimilations were by diffusion mechanism. The M cations in high quantities blocked the diffusion of other cations in seedlings. As can be seen from figures 2-6, all seedlings treated with ML solutions increased. In conclusion, minor increases in ML concentrations stimulated development of seedlings. The inhibition degree of the seedlings growth increased at higher concentration than 0.1 ppm and depended by the toxicity of M cations. The slow increasing of M concentrations in seedlings were due to their coordinations in polyoxometallate units. On the other hand, the association of M cations at polyoxometalates facilitated M transports in seedlings.

Conclusions

From the results it was observed that the series of Keggin polyoxometalates increased the efficiency of germination, except samples using Cu(II) complex solutions, which decreased slightly the germination efficiency.

The monolacunary polyoxoanion $K_2[\text{PVW}_{10}\text{O}_{39}]$, had the ability to act as a polydentate ligand for transition metal ions, so it was able to stimulate the growth of seedlings. The action of $K_2[\text{PVW}_{10}\text{O}_{39}]$ stimulated the increasing of the germination capacity, the anatomical parts of plants. In conclusion increasing the concentration of $K_2[\text{PVW}_{10}\text{O}_{39}]$ solutions stimulated the yield of biomass and seedlings growth.

The Cu (II) complex polyoxometalate, inhibited the Triticale seeds germination and even further development of seedlings. The inhibition of seminal roots development can be attributed to the toxic action of Cu (II) ions released from the Cu(II) polyoxoanion complex, which is set mainly in the roots [23]. The biomass accumulation has significant increase in using 1 ppm Cu(II) complex solution, where the root system [23]. The growth stimuli of the radicles and the seminal roots from treatment with Co(II) complex solutions was due to release in free Co (II) cations, which is set mainly in the root system [23].

Ni(II) complex solutions at low concentrations (0.1-1 ppm) stimulated the root growth, while at 10 ppm stimulated the coleoptile growth. This behaviour may be due to the fact that nickel ions are well absorbed in the whole plant [23].

According to the literature, the Mn (II) cations were established in all parts of seedlings and stimulated their growth significantly only in 0.1 ppm Mn compounds solution.

The significant increase of the radicles when the Triticale seeds were treated with $K_2[\text{FePVW}_{10}\text{O}_{39}]_2[H_2O]$, was attributed to Fe(III) ions released from the complex, especially in 0.1 ppm concentration [23].

In conclusion, the best fertilizer from all used compounds with 0.1-100 ppm concentrations was the free polyoxoanion ($K_2[\text{PVW}_{10}\text{O}_{39}]_2$), without metallic cations coordinated, having similar behaviour with EDTA chelated ligand widely used [29].

<table>
<thead>
<tr>
<th>Concentrations of ML solutions</th>
<th>Control</th>
<th>0.1 ppm</th>
<th>1.0 ppm</th>
<th>10 ppm</th>
<th>100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of M cations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(II) from CuL treatment</td>
<td>4.3503</td>
<td>4.573</td>
<td>5.8075</td>
<td>7.7812</td>
<td>11.175</td>
</tr>
<tr>
<td>Co(II) from CoL treatment</td>
<td>0.3068</td>
<td>0.3932</td>
<td>0.569</td>
<td>1.4892</td>
<td>2.862</td>
</tr>
<tr>
<td>Ni(II) from NiL treatment</td>
<td>0.8939</td>
<td>0.9832</td>
<td>1.1544</td>
<td>1.701</td>
<td>3.8662</td>
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<tr>
<td>Mn(II) from MnL treatment</td>
<td>82.5764</td>
<td>82.7495</td>
<td>84.1456</td>
<td>91.7918</td>
<td>128.4996</td>
</tr>
<tr>
<td>Fe(II) from FeL treatment</td>
<td>58.4228</td>
<td>58.566</td>
<td>59.2138</td>
<td>65.0288</td>
<td>68.6984</td>
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</table>

Table 3

CONCENTRATIONS OF M CATIONS IN Triticale SEEDLINGS AFTER TREATMENT WITH KEGGIN POLYOXOANIONS SOLUTIONS

References


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