New Keggin Polyoxometalates with Mixed Addenda as Stimulators of *Triticale* Seedlings Growth and Biomass Production

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In this paper, six new Keggin polyoxometalates: $K_8[PVMo_{10}O_{39}]$ and $K_x[MPVMo_{10}O_{39}(H_2O)]$ (x = 6 for M = Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and x = 5 for M = Fe^{3+}) were synthesised and their solutions by 0.1 μM, 1.0 μM, 10 μM and 100 μM concentrations were used to Triticale seeds soaking. All experiments were performed in hermetically germinators, in dim and isolated room, at 22°C and 38% humidity. Germination yields in the fourth and sixth days, the growth of radical and seminal roots, coleoptile and first leaf and biomass production were determined. Germination yields of Triticale seeds were not significantly different between treatment with polyoxometalates solutions and control (distilled water). The growth of seedlings were easy stimulated at increasing of $K_8[PVMo_{10}O_{39}] \cdot 16H_2O$ concentrations. Heavy metal cations of $K_x[MPVMo_0O_{39}(H_2O)]$ stimulated the seedlings growth in order: Ni > Fe > Mn > Cu > Co. Concentrations of heavy metals in Triticale seedlings were determined to establish relationship between their bioaccumulation and inhibitory action. The chelating action of $K_8[PVMo_{10}O_{39}]'"16H_2O$ and the interaction between heavy metals and molybdenum atoms proved to have an important role.

Keywords: Keggin, polyoxometalates, germination, Triticale

Keggin polyoxometalates are inorganic polycondensation compounds [1, 2]. Until now, polyoxometalates with mixed addenda atoms were used as catalysts in homogeneous and heterogeneous catalytic oxidation of organic compounds but also in biochemical reactions [3 - 7]. The presence of vanadium atoms in structures of Keggin polyoxometalates improved catalytic properties [4]. Redox properties of polyoxo-metalates with mixed addenda were used in the electrochemical multisensor manufacture [8]. Properties of polyoxometalates, as high basicity and solubility in water and organic solvents, recommended this to be used in chemical and biochemical analysis [7]. Polyoxometalates activity in enzymatic redox processes, as antitumoral, antibacterial and antiviral treatments was investigated and proved [7].

Few studies of polyoxometalates involvement in the plants biology were performed, mainly in enzymatic reactions and also in the delignification process [9-11]. Some elements are deficient in the soil, so it is necessary adding fertilizers. Polyoxometalates taken in this study contain macronutrients and micronutrients essential for growth and development of plants [12-14]. Moreover, monolacunary species, $K_8[PVMo_{10}O_{39}]$, has chelating ability to link a large variety of metal cations, as EDTA and other chelating compounds, widely used in fertilizer [15].

Keggin polyoxometalates with mixed addenda: $K_8[PVMo_{10}O_{39}]$ and its $K_x[MPVMo_{10}O_{39}(H_2O)]$ complexes (x = 6 for M = Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and x = 5 for M = Fe^{3+}) were synthesized. Solutions from 0.1μM to 100μM concentrations were prepared for everyone. In germinators, *Triticale* seeds were soaked with solutions of these compounds. Experimentally, the germination yield, the growth of the radical, seminal roots, the coleoptile and the first leaf were measured, the biomass productions were calculated and the heavy metals accumulation were determined for *Triticale* seedlings. Statistical interpretation of results was effectuated with T TEST function and with percentage differences [16]. T TEST function was used in determination of significant differences between control (distilled water) and samples, characterised by the probability parameter P, which must be under 0.05. Percentage differences (% differences) calculate with equation:

$$\% \text{ Difference} = (\bar{x}_{\text{sample}} - \bar{x}_{\text{control}}) \cdot 100 / \bar{x}_{\text{control}} \quad (1)$$

where: $\bar{x}_{\text{sample}}$ and $\bar{x}_{\text{control}}$ are arithmetic averages of sample, respectively of control.

Germination of *Triticale* seeds was studied by the authors, using Keggin polyoxometalates with tungsten ions instead of molybdenum and results were reported before [16].

Experimental parts

Materials and methods

All chemicals used in synthesis were obtained from commercial sources and they were at least of analytical purity. Distilled water used in all procedures. Elemental analysis was performed with a Varrian ASA 220 type spectrophotometer. Potassium was determined by FEP with an Eppendorf flame photometer. Thermal stability analyses were carried out on a Paulik-Erdely OD-103 derivatograph (20-800°C) at 5°C min^{-1}. FT-IR spectra were recorded in 400-4000 cm^{-1} range on a Biorad FTS 60A spectrophotometer using KBr pellets. Raman spectra were performed on solid powders at room temperature, using a DILOR OMARS 89 Raman spectrophotometer (λe = 1064 nm). UV-VIS spectra were recorded in 190-1100 nm range on Shimadzu UV-VIS model mini-1240 spectrophotometer.

T TEST function from Excel 2007 software was used for statistical interpretation of results.

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*Synthesis of $K_8[PVMo_{10}O_{39}] . 16H_2O$ (**L**)*

A mixture between 12.1 g (50.00 mmol) $Na_2MoO_4 . 2H_2O$, 0.7 g (5.0 mmol) $NaH_2PO_4 . H_2O$ and 1.00 g (5.0 mmol) $NaVO_3 . 4H_2O$ was solved in minimum quantity of distilled water under vigorous stirring. The *p*H value was adjusted with HCl solution to 4.7. The final solution was filtrated and in the red filtrate were added 4.00 g (54 mmol) KCl. Orange crystals of $K_8[PVMo_{10}O_{39}] . 16H_2O$ started to create at 5°C. Crystals were collected, washed and dried in a desiccator. Yield: 5.1 g (45 %). UV (nm): 212; 310; IR (cm^{-1}): 3569 s, 3489 s, 3474 s, 3186, 1624 m, 1042 w, 1031 w, 984 m, 945 vs, 929 s, 839 m, 807 m, 729 m, 690 sh, 593 w, 523 w; Raman (cm^{-1}): 960 vs, 889 w, 505 w, 376 w, 229 m, 150 w; Anal. Calcd: K, 13.80; Mo, 42.34; V, 2.25; P, 1.37; H_2O, 12.71. Found: K, 14.00; Mo, 42.00; V, 2.00; P, 1.50; H_2O, 12.5%.

*Synthesis of the $K_6[Mn(H_2O)PVMo_{10}O_{39})] . 10 H_2O$ (**1**)*

2.27 g (1.0 mmol) $K_8[PVMo_{10}O_{39}] . 16H_2O$ were added under vigorous stirring to a solution obtained by dissolving of 0.2 g (1.0 mmol) $MnCl_2 . 4H_2O$ in 25 mL distilled water at 4.3 *p*H, continuous adjusted with 0.1 N HCl. A clear red-brown solution was obtained and 1 g (13.4 mmol) KCl was added. The solution was kept for 2-3 days at 6°C and brown-orange crystals of $K_6[Mn(PVMo_{10}O_{39})(H_2O)] . 10H_2O$ were formed, filtered and washed with ethanol. Yield: 1.12 g (52 %). UV (nm): 217; 313.5; IR (cm^{-1}): 3564 m, 3378 m, 1616 m, 1079 sh, 1062 w, 1045 w, 945 sh, 932 s, 870 m, 782 m, 720 m, 680 m, 643 m, 522 w; Raman (cm^{-1}): 1095 w, 987 s, 885 w, 229 m; Anal. Calcd for $K_6[Mn(H_2O)PVMo_{10}O_{39}] . 10H_2O$: K, 10.90; Mo, 44.56; V, 2.37; Mn, 2.55; P, 1.44; H_2O, 9.20. Found: K, 10.84; Mo, 44.72; V, 2.20; Mn, 2.47; P 1.43; H_2O, 9.875%.

*Synthesis of the $K_5[Fe(H_2O)PVMo_{10}O_{39}] . 8H_2O$ (**2**)*

The synthesis procedure above was followed using 0.16 g (1.0 mmol) $FeCl_3$ instead of $MnCl_2 . 4H_2O$. Yield: 2.85 g (60.15 %). UV (nm): 218, 312; IR (cm^{-1}): 3470 m, 1609 m, 1080 w, 1068 w, 1051 w, 944 s, 858 m, 776 vs, 593 w; Raman (cm^{-1}): 995 s, 975 sh, 233 m; Anal. Calcd for $K_5[Fe(PVMo_{10}O_{39})(H_2O)] . 8H_2O$: K, 9.38; Mo, 46.18; V, 2.45; Fe, 2.69; P, 1.49; H_2O, 7.79. Found: K, 9.30; Mo, 46.31; V, 2.29; Fe, 2.58; P, 1.42; H_2O, 7.81%.

*Synthesis of the $K_6[Co(H_2O)PVMo_{10}O_{39}] . 22H_2O$ (**3**)*

The synthesis procedure above was followed using 0.238 g (1.0 mmol) $CoCl_2 . 6H_2O$ instead of $MnCl_2 . 4H_2O$. Yield: 1.21 g (51 %). UV (nm): 212, 315; IR (cm^{-1}) 3470 sh, 3373 m, 1617 m, 1080 w, 1068 w, 1051 w, 942 s, 887 m, 779 s, 520 w; Raman (cm^{-1}): 989 s, 974 s, 229 m; Anal. Calcd for $K_6[Co(PVMo_{10}O_{39})(H_2O)] . 22H_2O$: K, 9.88; Mo, 40.45; V, 2.15; Co, 2.47; P, 1.3; H_2O, 17.45. Found: K, 10.0; Mo, 42.2; V, 2.1; Co, 2.4; P, 1.3; H_2O, 17.2%.

*Synthesis of $K_6[Ni(H_2O)PVMo_{10}O_{39}] . 21H_2O$ (**4**)*

The synthesis procedure above was followed using 0.238 g (1.0 mmol) $NiCl_2 . 6H_2O$ instead of $MnCl_2 . 4H_2O$. Yield: 1.37 g (58 %). UV (nm): 214, 314; IR (cm^{-1}): 3569 sh, 3470 sh, 3373 m, 1616 m, 1080 sh, 1062 w, 1045 sh, 945 sh, 939 vs, 874 s, 788 vs, 645 w, 518 w, 225 w; Raman (cm^{-1}): 991 s, 975 sh, 235 m; Anal. Calcd for $K_6[Ni(PVMo_{10}O_{39})(H_2O)] . 21H_2O$: K, 9.96; Mo, 40.775; V, 2.16; Ni, 2.49; P, 1.32; H_2O, 16.82. Found: K, 9.89; Mo, 40.89; V, 2.02; Ni, 2.41; P, 1.28; H_2O, 16.72%.

*Synthesis of $K_6[Cu(H_2O)PVMo_{10}O_{39}] . 17H_2O$ complex (**5**)*

The synthesis procedure above was followed using 0.17 g (1.0 mmol) $CuCl_2 . 6H_2O$ instead of $MnCl_2 . 4H_2O$. Yield: 1.08 g (47 %). UV (nm): 211, 316; IR (cm^{-1}): 3569 sh, 3373 m, 1617 m, 1080 w, 1062 w, 1045 w, 941 s, 872 m, 782 s, 593 w, 520 sh; Raman (cm^{-1}): 996 sh, 979 vs, 519 w, 235 m, 156 w, 108 w; Anal. Calcd for $K_6[Cu(PVMo_{10}O_{39})(H_2O)] . 17H_2O$: K, 10.26; Mo, 41.94; V, 2.23; Cu, 2.78; P, 1.36; H_2O, 14.16. Found: K, 10.20; Mo, 42.15; V, 2.18; Cu, 2.62; P, 1.33; H_2O 14.11%.

Samples preparation

Triticale seeds were used in all experiments, 94% germination capacity and 14.4% humidity. Seeds were washed with distilled water, dried and sorted before using in experiments. Five solutions by 0.1 μM, 1.0 μM, 10 μM and 100 μM concentrations were prepared for **L** and **1 - 5** complexes, too. The control used was distilled water. The *p*H values were around 6.0 for all solutions used in experiments.

*Experiments using **L** solutions*

Triticale seeds were placed in a germinator, on a filter paper soaked with 25 mL 0.1 μM **L** solution. The germinator was closed and put in a dim and isolated room, at 22°C and 38% humidity. In the fourth day, the germinator was opened and germinated seeds were counted. The filter paper was soaked again with 25 mL 0.1 μM **L** solution. The germinator was shut and kept again two days, in the same conditions, mentioned before. In the sixth day, the germinated seeds were counted and seedlings were measured. Seedlings were weighed and dried in a Thermo Heraeus oven for biomass determination. All steps of experiment were followed in the same time and condition for control (distilled water) and 1μM, 10μM and 100μM **L** solutions.

*Experiments using **1** solutions*

Preparation of *Triticale* seeds for germination in 0.1 to 100 μM **1** solutions was performed in the same way as in the experiments with **L** solutions. In parallel a germinator with distilled water as control was prepared. Storage conditions of germinators and the procedure for effecting measurements were the same as in the experiments with **L** solutions. Seedlings growth and the biomass were determined. Seedlings dried were digested and manganese concentrations were determined using AAS method.

*Experiments using **2** solutions*

The same technique from **L** experiments was used to prepare germinators with *Triticale* seeds wetted with control, respectively 0.1 μM, 1.0 μM, 10 μM and 100 μM complex **2** solutions. Elongations, biomasses and iron concentrations were determined for sprouted seeds.

*Experiments using **3** solutions*

Experiments were conducted following the steps presented in experiments of **L** solutions, using **3** solutions in the same concentrations. Seedling elongations were measured, biomass production was determined after dry and cobalt concentrations were calculated after digestion and AAS determination.

*Experiments using **4** solutions*

Triticale seeds were put to germination in the same conditions as in the first experiments with **L** solutions, but using control, respectively 0.1 μM, 1.0 μM, 10 μM and 100 μM **4** solutions. Seedling were measured, dried and digested for determination of elongations, biomasses and nickel concentrations.

*Experiments using **5** solutions*

Experiments were developed in the same way as first experiments with **L** solutions, but using control, respectively 0.1 μM, 1.0 μM, 10 μM and 100 μM **5** solutions. Seedling were measured, dried and digested for determination of elongations, biomasses and copper concentrations.

Statistical interpretation of results was achieved by P calculation with TTEST and by plotting of percentage differences for each parameter in all experiments performed.

Results and discussions

Germination yields of *Triticale* seeds in the fourth day and in the sixth day were recorded in table 1. In the sixth day, yields were above 75% in all experiments and were higher than in the fourth day of germination. Generally, germination yields were greater in experiments with polyoxometalates solutions than in control, in the same experimental conditions. Differences between treatment of *Triticale* seeds with control and polyoxometalates solutions were insignificant in all experiments effectuated, proving that the germination is largely due to seminal reserves. The highest yield of germination was realized after using 100 μM **1** solution (92%). Also, the number of germination seeds increased significantly under treatment with 10 μM **3** and 10 μM **4**, where the germination yield was 90%. The lowest germination yield of *Triticale* seeds was 75% in 1.0 μM **3** and 0.1 μM **4** solutions.

Seedlings parameters and the biomass production were determined in all experiments. Significant differences ($P < 0.05$) between seedling parameters after the treatment with control and polyoxometalates solutions were determined with TTEST EXCEL 2007 function and were noted in table 2. Significant differences in growth of radicles were recorded only treatments with 0.1 μM0**L**, 1.0 μM **3**, 100 μM **4**, 10 μM **1** and 100 μM **2**. Seminal roots elongations were significantly influenced, especially in treatments with 0.1-100 μM **3**, but also in using of 100 μM **L**, 100 μM **1** and 10 μM **2** solutions, 0.1 and 100 μM **4**, 10 and 10 μM **5** solutions. The coleoptile growth was significantly affected by using of 0.1-100 μM **3** and **4** solutions, but also by 0.1-1.0 μM **1** and 10- 100 μM **5** solutions. Significant differences between control and polyoxometalates in increasing of first leaf were recorded in 10 and 100 μM **L** solutions, 0.1 μM **1** solution, 1.0 and 10 μM **3** solutions, 100 μM **4** and 10 μM **5** solutions. The biomass production varied significantly after watering of *Triticale* seeds with **3** and **5** solutions. Other solutions with significant influence of the biomass production were 0.1 and 1.0 μM **L**, 10 μM **1**, 0.1 μM **2** and 100 μM **4** solutions.

Influence of **L** solutions in the growth and the development of *Triticale* seedlings, comparatively with control, was showed in Figure 1.a. The growth of radicles was inhibited in treatment with 0.1 μM **L** (-15.74%) and 1.0 μM **L** (-10.65%) and was stimulated with 8.87% after watering with 100 μM **L** solution. The elongation of seminal roots was inhibited with 10.24% after soaking of *Triticale* seeds in 0.1μM **L** solution and was stimulated in 1.0 -100 μM **L** solution, with maximum 11.70% in 100 μM **L**. The coleoptile growth wasn't influenced significantly by all **L** solutions using in experiments. The growth of first leaf was stimulated by all **L** solutions, especially in the presence of 10 μM (15.43%) and 100 μM (21.48%) solutions. Increasing of the biomass production was stimulated by 0.1 μM and 1.0 μM **L** solutions and it was greater by 1.0 μM **L** solution

Polyoxometalate type	Germination yield (%)									
	Control		0.1 μM		1.0 μM		10 μM		100 μM	
	4th day	6th day	4th day	6th day	4th day	6th day	4th day	6th day	4th day	6th day
L	82	82	81	88	82	82	80	80	88	88
1	82	82	84	88	82	82	80	80	88	92
2	80	86	76	78	83	84	82	84	85	85
3	83	83	71	78	75	75	90	90	84	84
4	82	86	75	75	86	88	87	90	80	86
5	76	80	87	87	84	88	82	84	81	84

Table 1
GERMINATION YIELDS OF *TRITICALE* SEEDS SOAKED WITH CONTROL, **L** AND **1 – 5** SOLUTIONS

Polyoxometalate type	Concentration	Probability parameter - **P**				
		Radicles	Seminal roots	Coleoptile	First leaf	Biomass
L	0.1 μM	*0.04*	0.08	0.34	0.19	*< 0.05*
	1.0 μM	0.13	0.29	0.28	0.08	*0.02*
	10 μM	0.27	0.31	0.23	*0.02*	0.25
	100 μM	0.16	*0.04*	0.35	*< 0.01*	0.17
1	0.1 μM	0.44	0.37	*< 0.05*	*0.01*	0.24
	1.0 μM	0.08	0.47	*0.02*	0.08	0.33
	10 μM	*0.03*	*0.03*	0.11	0.10	*< 0.01*
	100 μM	0.48	*< 0.05*	0.22	0.45	0.28

Table 2
DETERMINATION OF THE PROBABILITY PARAMETR , **P** - USING TTEST EXCEL 2007 FUNCTION

2	0.1 μM	0.16	0.18	0.31	0.16	0.38
	1.0 μM	0.21	0.20	0.25	0.50	0.43
	10 μM	0.36	*0.04*	0.35	0.38	0.11
	100 μM	*< 0.05*	0.10	0.16	0.30	*0.01*
3	0.1 μM	0.18	*0.01*	*< 0.05*	*< 0.05*	*< 0.01*
	1.0 μM	*0.01*	*< 0.01*	*< 0.01*	*0.01*	*< 0.01*
	10 μM	0.27	*0.01*	0.07	0.26	0.19
	100 μM	0.15	*0.01*	*0.03*	0.27	*0.01*
4	0.1 μM	0.39	*0.03*	*< 0.01*	0.08	0.37
	1.0 μM	0.10	0.07	*< 0.01*	0.20	0.33
	10 μM	0.36	0.07	*0.01*	0.41	0.18
	100 μM	*< 0.05*	*0.01*	*< 0.01*	*0.03*	*< 0.01*
5	0.1 μM	0.45	0.25	0.09	0.38	*0.02*
	1.0 μM	0.50	0.28	0.12	0.16	*0.01*
	10 μM	0.40	*0.05*	*< 0.01*	*0.02*	*0.03*
	100 μM	0.44	0.29	*0.01*	0.47	*0.04*

(9.29%). The biomass production of seedlings started to inhibit by 10 μM and 100 μM **L** solutions.

In figure 1.b., the influence of 0.1 - 100 μM **1** solutions on the growth of *Triticale* seedlings was exposed. The radicle growth was inhibited by 1 μM and 10 μM **1** with more 10%, while their growth was not affected by 0.1μM and 100 μM **1** solutions. The growth of seminal roots was inhibited by 10 μM **1** (-11.99%), was slightly stimulated in 100 μM **1** (8.09%) and was unmodified in 0.1 - 1.0 μM **1** solutions. The treatment with 0.1-10 μM **1** inhibited slightly the growth of coleoptile from -7.47% to -4.36%. The growth of first leaf was more inhibited than coleoptiles growth by using of 0.1 - 10 μM **1** solutions. The lowest elongation of the first leaf (11.97%) was recorded in 0.1 μM **1** solution. The inhibition of first leaf growth declined with increased of the **1** concentration from -8.03% in 1.0 μM to -7.74% in 10 μM. The first leaf growth was not disturbed by 100 μM **1** solution. The biomass production was significantly (11.42%) only in 10 μM **1** solution**,** and was less influenced in other **1** solutions.

Percentage differences between control and 0.1 - 100 μM **2** solutions were recorded in figure 1.c. The radicle growth was stimulated generally from 7.65% by 1μM **2** to 15.41% 100μM **2**, but was not affected by 10 μM **2** solution. The growth of seminal roots was inhibited by all 0.1 - 100 μM **2** solutions and the lowest elongations were recorded in 10 μM **2** solution (-9.64%). The growth of coleoptile and first leaf were less influenced by 0.1-100 μM **2** solutions and the largest increase of the first leaf was 5.12% in 0.1 μM solution. The biomass production was inhibited until 10.64% by 100 μM **2** solutions and was unmodified in 0.1 - 1.0 μM **2** solutions.

Generally, growth of *Triticale* seedlings was inhibited after using of 0.1 - 100 μM **3** solutions,as can be seen in figure 1.d. Most inhibitory action on the growth of seedlings showed 1.0 μM **3** solution. 1.0 μM **3** solution inhibited radicles growth with 22.96%, growth of seminal roots with 23.91%, coleoptile growth with 9.80% and the growth of first leaf with 16.96%. However, the biomass production increased in all experiments treated with 0.1 - 100 μM **3** solutions. Important increase of biomass were recorded at watering with 0.1 μM (15.44%) and 1.0 μM (13.66%) **3** solutions.

In figure 1.e., the involvement of 0.1-100 μM **4** solutions in growth of *Triticale* seedlings was showed. Radicle elongations increased significantly in 1.0 μM **4** (13.09%) and 100 μM **4** (14.57%). The growth of seminal roots was ranged from 11.70% in 10 μM **4** to 20.19 % in 100 μM **4**, and the differences between the action of 0.1 μM, 1.0 μM and 10 μM **4** were insignificantly. The highest coleoptile elongation of *Triticale* seedlings was recorded in 0.1 μM **4** (21.68%) and was only slightly modified in other solutions, from 10.97% in 10 μM **4** to 13.78% in 100 μM **4**. The first leaf elongation increased significantly only in 100 μM **4** (14.04%). A growth of the first leaf over 10% was recorded in 0.1 μM **4** solution. The biomass production of *Triticale* seeds was inhibited slowly by with 0.1-10 μM **4** solutions and was inhibited strong by 100 μM **4** solution. The lowest value of biomass recorded after using of 100 μM **4** solution was -14.04%. Percentage differences of biomasses from experiments with 0.1-10 μM **4** solutions were very close, from -1.68% to -4.65%.

In figure 1.f., it can be seen that biomass of *Triticale* seedlings was stimulated by treatment with **5** solutions at absolutely all concentrations taken in study. The biomass production after watering of *Triticale* seeds with **5** solutions increased from 9.59% in 100 μM to 12.24% in 0.1 μM **5**. Radicles growth of *Triticale* seedlings wasn't influenced by 0.1 - 100 μM **5** solutions. The growth of seminal roots was significantly inhibited only after soak in 10 μM **5** (-10.83%), while the growth of coleoptile was inhibited significantly in 10 μM **5** (-10.40%). Both, 1.0 μM and 10 μM **5** solutions inhibited the growth of first leaf from 5.63% to 11.90%.

In figure 2.a, it can see that radicles growth was stimulated by **2** solutions of iron complex, at all concentrations, especially at 100 μM. Both, 100 μM solutions of **L** and **4** stimulated the radicles growth. On the

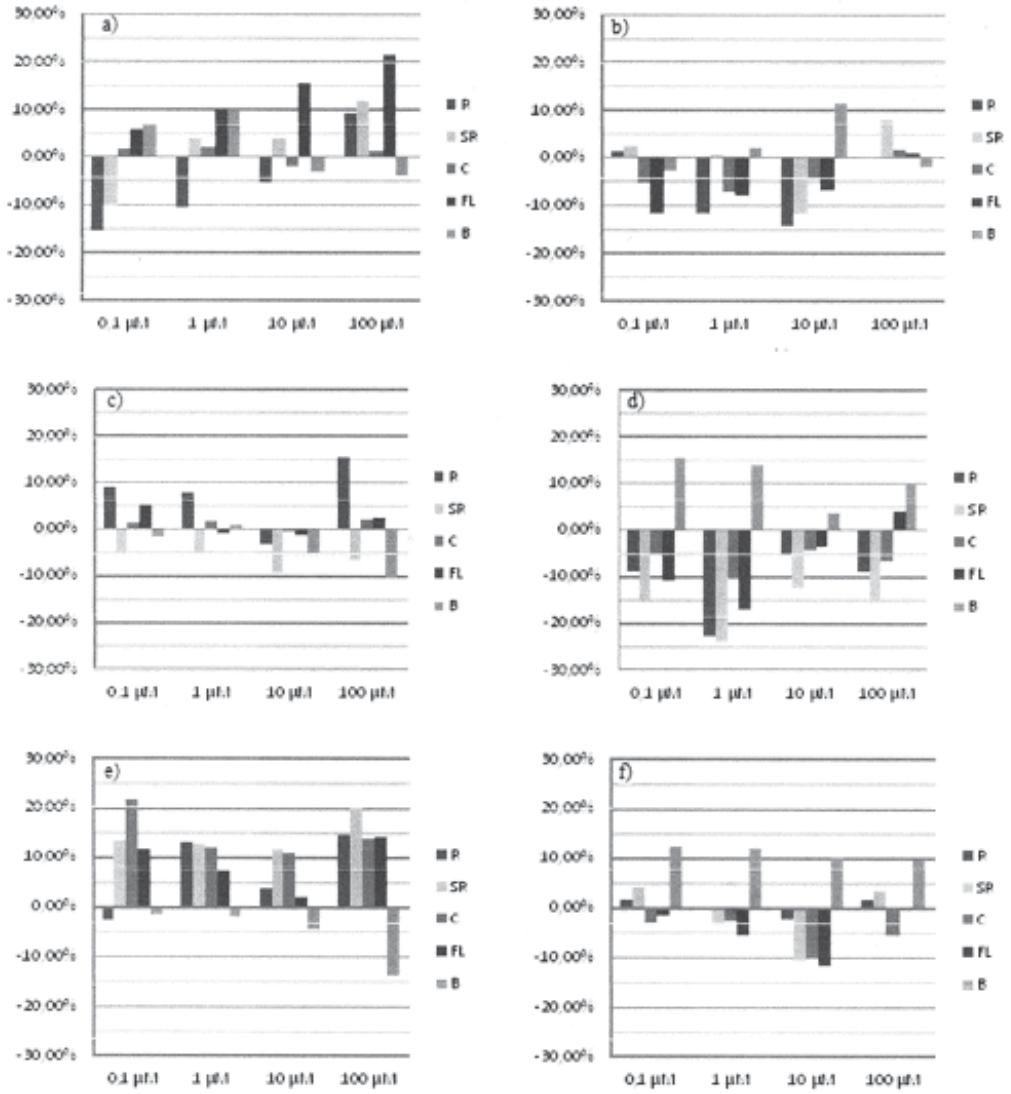

Fig. 1. Percentage differences between biological parameters of *Triticale* seedlings after sprinkling with a) **L** solutions; b) **1** solutions; c) **2** solutions; d) **3** solutions; e) **4** solutions; f) **5** solutions; (R-radicle, SR- seminal roots, C-coleoptile, FL-first leaf, B - biomass).

other hand, **3** solutions containing cobalt cations inhibited radicles growth, mainly that of 1.0 μM. More, inhibitory action was exhibited in 0.1 - 10 μM **L** and 1 - 10 μM **1** solutions.

The growth of seminal roots was stimulated by **4** solutions of nickel complex (fig. 2.b.). Significantly growth of seminal roots resulted after use 1.0 and 100 μM **4** solutions. The highest growth of seminal roots was recorded after treatment with 100μM **2** solution. The seminal roots growth was stimulated too by 100 μM **L** and **1** solutions. A strong inhibitory activity was displayed by 0.1 μM **L**, 10 μM **1** and 1.0 μM **3**.

In figure 2.c., it was observed that only **4** solutions stimulated coleoptiles growth, between 10 -20% at 1.0 - 100μM and over 20% at 0.1 μM. The coleoptiles growth was inhibited by all **3** and **5** solutions, especially at 1.0 and 10 μM. The strongest inhibitory activity was manifested by 10 μM **5** solution. Solution **1** inhibited coleoptiles growth at low concentrations (0.1 - 10 μM). The influence of **L** solutions on coleoptiles growth was insignificant.

Only **4** solutions stimulated the growth of first leaf in whole range of concentrations. The highest growth of first leaf was accomplished by 100 μM **4** solution (fig. 2.d.). The first leaf growth was stimulated by all studied polyoxometalates solutions by 100 μM, except the solution of **5** complex. The elongation of leaf was inhibited over 10% by 0.1 μM **L** and **3** solutions, respectively 10 μM **5** solution. The strongest inhibitory action of the first leaf growth was over 15% after the treatment with 1.0 μM **3** solution.

Biomass production was stimulated by **L**, **3** and **5** solutions, especially at lower concentrations, while at high concentration (100 μM), biomass production was inhibited by **2** and **4** solutions (fig. 2.e.).

In table 3, concentrations of heavy metals determined with AAS method in *Triticale* seedlings were recorded. Concentrations of all heavy metal cations were found under toxicity limits reported by other authors [17]. The concentration of manganese ions in *Triticale* seedlings had an important increase only after using of 100 μM **1** solution (97.9514 μg g^{-1}) and at this concentration the seedlings growth started to be stimulated, as reflected in figure 1.b. Manganese concentrations accumulated in seedlings after treatment with 0.1 - 10 μM **1** solutions, from 82.7495 to 87.0753 μg g^{-1}, slightly inhibited the growth of seedlings. The increasing of manganese concentration was insignificant in all experiments. Results confirmed that the growth of *Triticale* seedlings became important at higher manganese concentrations and this fact was mentioned before in literature [18, 19]. Iron concentrations (table 3) varied in a narrow range from 55.8933 μg g^{-1} in control to 68.867μg g^{-1} in 100μM **2**. From association between table 3 data and figure 1.c. graphs can be concluded that the stimulation of radicles elongation and the inhibition of biomass production were observed at 68.867 μg g^{-1} iron after using of 100 μM **2** solution. The stimulation of seedlings growth in the presence of **2** can be affected by

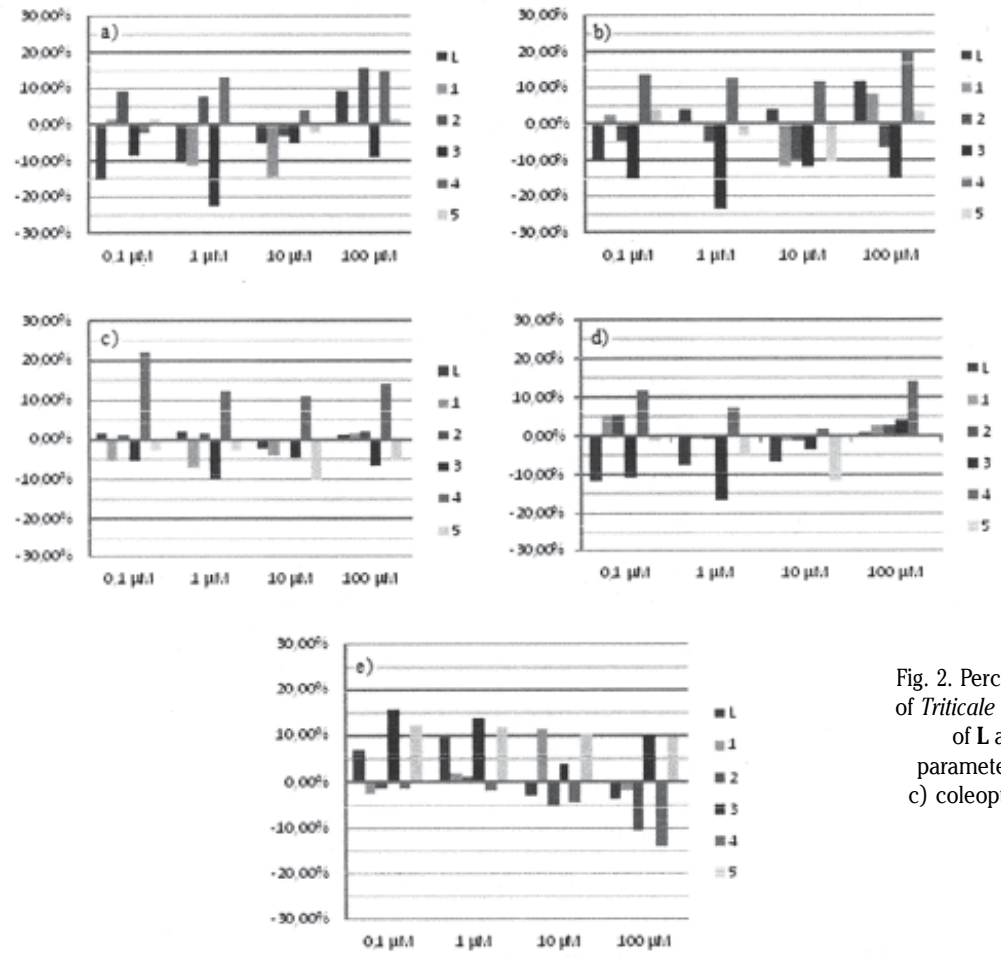

Fig. 2. Percentage differences after treatments of *Triticale* seedlings with 0.1-100µM solutions of **L** and **1-5** for the next biological parameters: a) radicles; b) seminal roots; c) coleoptiles; d) first leaves; e) biomasses

Concentration of heavy metal cations in seedlings ($\mu g\ g^{-1}$ dry weight)	Concentrations of **1-5** complex solutions				
	Control	0.1 µM	1.0 µM	10 µM	100 µM
Manganese after using **1** solutions	82.5764	82.7495	83.2735	87.0753	97.9514
Iron after using **2** solutions	55.8933	56.0982	56.6695	61.1315	68.867
Cobalt after using **3** solutions	0.3068	0.4212	0.6342	1.169	3.6973
Nickel after using **4** solutions	0.9374	1.0542	1.325	1.812	2.5963
Copper after using **5** solutions	4.289	4.5912	5.0044	8.9204	11.6938

Table 3 CONCENTRATIONS OF HEAVY METAL CATIONS FOUND IN *TRITICALE* SEEDLINGS AFTER TREATMENT WITH **1-5** KEGGIN COMPLEXES SOLUTIONS, USING AAS METHOD

the interaction between the reduction process of Fe^{3+} to Fe^{2+} with molybdate and phosphate [20]. A broad concentration range (0.3068 -3.6973 $\mu g\ g^{-1}$) was obtained for cobalt ions in seedlings after treatment with control and 0.1 - 100 µM **3** solutions (table 3). From table 3 data and figure 1.d. representations can conclude that growth of *Triticale* seedlings was slowed at all cobalt concentrations and became very sensitive at 0.6342 $\mu g\ g^{-1}$ cobalt. Instead, the biomass production was favoured mainly by 0.4212 - 0.6342 $\mu g\ g^{-1}$ cobalt accumulation in 0.1 - 10 µM **3** solutions. Large differences between the behaviour of *Triticale* seeds treated in cobalt complexes with molybdenum and respectively with tungsten can be due to the differences between the nature and the behaviour of two polyoxoemetalate ligands [16]. Nickel concentration was 0.9374 $\mu g\ g^{-1}$ in seedlings soaked in control and was ranged in 1.0542 - 2.5963 $\mu g\ g^{-1}$ in seedlings resulted from experiments with 0.1 -100 µM **4** solutions (table 3). All nickel concentrations determined in *Triticale* seedlings were in tolerance limit of plants and the stimulation of seedlings growth was possible in whole concentration range of **4** taken in study (fig. 1.e.) [21, 22]. In table 3, the copper ions concentration in *Triticale* seedlings did not increase more in 0.1-1.0 µM **5** solutions (4.5912 and 5.0044 $\mu g\ g^{-1}$) than in control (4.289 $\mu g\ g^{-1}$), but was significantly increased in 10 µM (8.9204 $\mu g\ g^{-1}$) and in 100 µM (11.6938 $\mu g\ g^{-1}$). Both, graphs of figure 1.f. and copper concentrations of table 3 showed a significant inhibition of seedlings growth at 10 µM **5** and an insignificant influence at 100 µM **5** solution, in spite of the high complexity of copper mechanism in plants [20, 23].

Conclusions

The free ligand, $K_8[PVMo_{10}O_{39}] \cdot 16H_2O$, did not stimulate significantly growth of seedlings, but a positive influence was observed with increasing of concentration. Linking of heavy metal cations produced different influences in *Triticale* seedlings development and stimulated the growth

of seedlings manifested in the next order: Ni > Fe > Mn > Cu > Co. Seedlings growth was inhibited by 0.1 - 10 μM $K_6[Mn(PVMo_{10}O_{39})(H_2O)] . 10H_2O$ and was stimulated significantly at 100 μM. This behaviour of $K_6[Mn(PVMo_{10}O_{39})(H_2O)] . 10H_2O$ can be due to the *p*H solution, the slowly mobility of manganese ions and the chelating effect of $K_8[PVMo_{10}O_{39}]$ ligand. The iron complex, $K_5[Fe(PVMo_{10}O_{39})(H_2O)] . 8H_2O$, stimulated especially the growth of radicles and first leaf, while the concentration of iron ions in seedlings was slightly variable in 0.1 - 100 μM range [18, 19]. $K_6[Co(PVMo_{10}O_{39})(H_2O)] . 22H_2O$ solutions were the best in biomass production, but in the same time generated the most inhibitory action in elongations of coleoptile and seminal roots, mainly in 1.0 μM solution. The complex of nickel, $K_6[Ni(PVMo_{10}O_{39})(H_2O)] . 21H_2O$ was the most efficiently in *Triticale* seedlings growth in whole concentration range taken in study, considering that the nickel toxicity is intermediary between heavy metals ions involved in these experiments [21, 22]. Stimulation of *Triticale* seedlings growth by nickel complex can be due to a good tolerance mechanism of seedlings to nickel and to a very good mobility of nickel in seedlings tissues [24]. Copper complex, $K_6[Cu(PVMo_{10}O_{39})(H_2O)] . 17H_2O$, inhibited seedlings growth only in 10μM solution, but stimulated biomass production. This particular behaviour in germination phase of *Triticale* seeds can be assigned to characteristic features of polyoxometalates complexes as the stability in aqueous solutions, the sensitivity to *p*H variation and to reducing agents. Comparing results from the watering of *Triticale* seedlings with polyoxometalates solutions of similarly compounds, some with molybdenum and tungsten others, revealed that a special attention must be payed to different chelating action between $K_8[PVW_{10}O_{39}] . 15H_2O$ and $K_8[PVMo_{10}O_{39}] . 16H_2O$ and at possible interactions in biochemical reaction between heavy metal cations (Mn, Fe, Co, Ni and Cu) and molybdenum, respectively tungsten atoms.

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