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QUANTITY DETERMINATION OF MOLYBDENUM FROM *PISUM SATIVUM* PLANTS AND THE INFLUENCE OF HEAVY METAL TO CHEMICAL ELEMENTS ACCUMULATION

DETERMINAREA CANTITĂŢII DE MOLIBDEN DIN PLANTELE DE *PISUM SATIVUM* ŞI INFLUENŢA METALELOR GRELE ASUPRA ACUMULĂRII ELEMENTELOR CHIMICE

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Abstract. *The aim of this study was to test the pea plant as sentinel specie for the heavy metal molybdenum. Evaluation of soil quality after the molybdenum uptake by pea revealed the following results: Pea plant is a bioindicator that concentrates molybdenum with fast reaction to increasing concentrations in soil. Molybdenum had a positive effect concerning the plant growth (throughout all experimental process, pea plants treated with highest concentrated metal solution reached the largest dimensions). Accumulated molybdenum was directly proportional to increasing concentration of the applied solution to roots, stem, leaves and flowers of the experimental plants; however it resided in flowerpot soil too .In the leguminous roots where the nitroreductase and nitrogenese activity is increased, molybdenum content was much higher compared to the aerial parts of the plant. All the way through molybdenum accumulation in the experimental plants up to high concentrations, other chemical elements revealed lower concentration although within the normal limits, with the exception of phosphorus. These plants were found to assimilate high molybdenum quantities without any detrimental consequences for them since molybdenum accumulation occurred in vacuoles in innocuous chemical forms.*

Key words: molybdenum, *Pisum sativum*, biomonitoring, soil, sentinel specie

Introduction

When categorizing plants that can grow in the presence of toxic elements, the terms "tolerant," "indicator", and "hyperaccumulator" are used. Tolerant specie is one that can grow on soil with concentrations of a particular element that are toxic to most other plants. While both indicator species and hyperaccumulators are

tolerant, studies have shown the genetic distinction of the mechanisms involved [1,2,3]. However, tolerant species are not necessarily indicators or hyper accumulators, as tolerant no accumulators can exclude metals from entering the root tissue. Indicator plants have been employed in biogeochemical prospecting. Molybdenum is better known since the demonstration of its relations with copper and sulphur in nutrition, although it has many biological functions like heavy metal in animal and vegetal regnum. In 1930 Bortels proved that molybdenum is essential in Azotobacter growth. Later was proved that all nitrogen fixation organisms and even superior plants need molybdenum. In 1930, Ferguson realized the connection between a severe diarrhea syndrome from Great Britain bovines and molybdenum excess. Richard proved the importance of molybdenum in 1935 and also Westerfeld (and in another part of the world by De Renzo) witch discover in the same time that xantinoxidase a flavoprotein is depending of molybdenum. Some science people sad that molybdenum has protection effect to teeth and that there will be a connection between molybdenum deficit and increasing number of dental caries and between molybdenum deficit and increasing number of renal diseases. From biological point of view is important Mo (V) and Mo (VI) states witch give anion complexes implicated in electrons transfer and nitrogen fixation [4,5,6]. Molybdenum has many and complex functions: is one of the essential elements for plant nutrition, it enters in some enzymes composition catalysed biochemical reactions, is implicated in atmospherically nitrogen fixation and nitrates reduction, influenced chlorophyll accumulation, in animal nutrition has also an important role by enzymatic activities [7]. There are differences concerning molybdenum needs between species and inside the specie. The most sensitive are clover and alfalfa, followed by *Cruciferae* and *Compositae* Families. Extremely sensitive to molybdenum are some vegetables like lettuce, spinach, tomatoes and red cabbage. Molybdenum concentration varieties at deficiency plants from 0.02 to 0.2 ppm. Species like vegetables have values of molybdenum concentration between 0.3 – 0.4 ppm. At plants in normal nutritional condition molybdenum concentration varying from 0.5 to 5 ppm. Because of molybdenum deficit the soils are high acid, sometimes with high levels of manganese, aluminium with bad effect to plants [8,9].

Materials and Methods

Experiment accomplish. Pea plant (*Pisum sativum*) was tested like "sentinel specie" for heavy metal molybdenum, because is well know that pea is a plant with selectivity for this metal [10]. The experiment evaluates soil quality using an active biomonitoring method, using pea plants treated with molybdenum chloride, at different concentration, chosen to not pollute soil use [11,12]. Seeds (pea seeds) germinated in a Petri recipient, being put uniformly to filter paper wet with solution used for treatment till the first root appear (32 ours – 42 ours to 20 ^{o}C, at dark). When root is small than 2 mm long, the seeds are planted in vegetation vessels at 10-mm underground [13,14]. Pea plants were planted in 66 vegetation

vessels (six vessels for every concentration of wetting solution) witch contains 800 g soil and were treated with a solution containing metallic ion Mo^{6+} from different concentration, and six vegetation vessels were water wet. This experience was during month March - May 2006, plants has moderate needs for heat. Growing develop in the same temperature and light conditions. After seeding, plants were daily treated with 10 ml solution containing Mo^{6+}. After a few days from emergence *Pisum sativum* plants started development, with the almost same dimensions and colour, indifferent by Mo^{6+} concentration, from solution used for treating plants. For verifying if soil quality was influenced by molybdenum chlorides introducing it get to the premise that soils of bad quality induce a significant reducing in root long comparing with a soil of quality. For this it will be measured the longest root at every plant and determine the media for all longest roots for every level of treatment. It will be compared medium long of roots treated with the ones from witness vessel [15]. Results are evaluated using standards [16].

Heavy metal water soluble in nitromuriatic acid from soil extraction. For heavy metal determination we weighed approximately1.5 g from soil sample with an 0.0001g exaction in a 100 ml reaction vessel. It wetting approximately 0.5- 1.0 ml water and it addition under mixture, 10 ml hydrochloric acid, then 5 ml nitric acid drop by drop if is necessary for reducing foaming. For 16 ours is left at room temperature for easy oxidation of organic part of soil, after this time it boils till drying. Nitromuriatic acid extraction must be realized under a well-ventilated hoot. Is essential to add boiling moderator granule at the witness and the other probes, for avoiding violent boiling and solution loss. After cooling reaction vessels, in soil samples add distillate water, filtering by filter paper and there are react with distillate water at 50 ml. Solutions obtained are prepared for determine zinc, iron, copper and molybdenum. For soil samples that contain more than 20% (m/m) organic carbon it must be treated with an extra quantity of nitric acid. Nitromuriatic acid is not totally dissolvent for the most of soils; the efficiency of extraction is different from a metal to another and is influenced by matrix compound. Metals extracted in nitromuriatic acid can't be considered total fractions, but also can't be consider bioaccessible fractions, because the extraction process is to power for representing a biological process [17].

Vegetal material preparation. Plants samples analyses for microelements and heavy metal determination is based to spectrometry measurement of atomic absorption at one element concentration in nitromuriatic acid extract. The plants were identified in the department of botany. Equipment used was compound for atomic absorption spectrophotometer-VARIAN SpectrAA 1100 and hydrure system VARIAN DGA 77. Mineralization: 5 grams mortared plants and sifting are introduced in a porcelain capsule. It adds 15 ml acids mixture (HNO_3: $HClO_4$: H_2SO_4 2:1:0.2), and then is boiled till evaporation. Operation repeating till the residue has white-yellow colour. The capsule let cooling at room temperature than there are react with hydrochloric acid (HCl 0.5 n) at 50 ml [18].

Microelements and heavy metals from plants determination. From plant samples it was determined heavy metal and microelements (Co, Fe, Mg) content

using an atomic absorption spectrophotometer. At every sample set there was a control sample for the reactive we used. For every determinate element it marks a calibration curve, after it determine the proper element from analysed samples. Determination method of microelements at atomic absorption spectrophotometer is respecting ISO 11466 [19].

Phosphorus content determination. Sample preparation: A part of vegetal ashes analytical weighed is wet with distillate water then add 40 ml HCl, it puts into a water bath and after 30 minutes it filters. The obtained solution is react at 100 ml. It takes 15 ml from solution, it put into a balloon of 100 ml adding 2.5 ml NH_4OH, 1 ml $(NH_4)_2MoO_4$ and 0.25 ml $SnCl_2$. It let rest until appears a blue coloration. Work mode: for determine phosphorus it marks a standard curve like in the next example: 0.199 g KH_2PO_4 is react to 250 ml. From the solution it take 10 ml witch react to 250 ml. From obtained solution it takes in 100 ml balloons 1,2, 3…9ml treated like analysed sample with molybdenum chloride and stannous chloride, after 15 minutes it colours. The solution from balloons will have concentrations between 0.01 and 0.09 mg P_2O_5 at 100 ml. Results: there are reading samples extinction, and from standard curve is read concentration levels of samples. All determine values are obtained by comparing to 100 g drying substance.

Results and Discussions

Plants use two methods to desorbs metals from the soil matrix: acidification of the rhizosphere through the action of plasma membrane proton pumps and secretion of ligands capable of chelating the metal. Plants have evolved these processes to liberate essential metals from the soil, but soils with high concentrations of toxic metals will release both essential and toxic metals to solution. Molybdenum chloride effects on *Pisum sativum* plant growth are presented in Table 1.

Table 1.

Molybdenum chloride effects to *Pisum sativum* plants

Nr.	Molybdenum concentration from solution (ppm)		
		Height (cm)	Length root (cm)
1	0	40	30.6
2	0.1	42	32.0
3	0.3	45	34.6
4	0.6	47	35.8
5	0.9	49	37.9
6	1.2	52	37.1
7	1.5	54	38.3
8	1.8	55	39.2
9	2.1	58	41.6
10	2.4	59	43.2
11	2.7	65	47.7
12	3	68	50

Analytical equation that describing plants height correlated with molybdenum chloride concentration is $y = 0.0519\ x^2 + 1.7303x + 38.773$, where correlation coefficient of *Pisum sativum* plants height for the 12 cases studied has the value $R^2 = 0{,}9875$. The dependence between the height of pea plant roots by molybdenum chloride concentration is described by the relation: $y = 0.0781\ x^2 + 0.555x + 31.164$, where correlation coefficient has the value $R^2 = 0.96$.

After 30 days from emerge it observe that plants treated with molybdenum solutions at high concentration are much more develop than the other one and had a greener colour. At the end of experiment, the differences between plants were visible. Plants treated with molybdenum solution in concentration higher than 3 ppm Mo^{6+}, 2.7 ppm Mo^{6+}, 2.4 ppm Mo^{6+} and 2.1 ppm Mo^{6+}, growing higher and had a greener colour than the one wet only with water and with solution containing molybdenum ion in smaller concentration of 0.1 ppm Mo^{6+}, 0.3 ppm Mo^{6+}, 0.6 and 0.9 ppm Mo^{6+}. *Pisum sativum* plants are vegetal indicators, from accumulation species category, witch retain molybdenum without exterior remarkable changes.

From Table 1 can be seen that at molybdenum concentration increment in wetting solution were increase the high of *Pisum sativum* plants but also root long length comparing with witness growth in same soil type, at the same temperature, light and humidity, but daily watering. In these conditions pea plants were between 40 cm (witness sample) and 68cm at plants treated with solution of 3 ppm Mo^{6+}.

This observation is valuable for *Pisum sativum* plants roots length, characteristic that grow proportional with molybdenum concentration from 30.6 cm at witness sample, to 50 at plants treated with solution containing higher molybdenum concentration ions. Although plants were wet with different concentrations of molybdenum chloride, pea plants develop normally comparing with witness sample growing in the same conditions but watering, how can be seen in Table 2.

Table 2.

Molybdenum content in root, stem, leaf and flower of *Pisum sativum* plants and it's soil

Nr.	Molybdenum concentration from solution (ppm)	Molybdenum content (ppm)				
		Soil	Root %.s.u.	Stem %.s.u.	Leaf %.s.u.	Flower .s.u.
1	0	3.5	4.3	1.6	1.2	0.22
2	0.1	3.5	4.7	1.8	1.7	0.5
3	0.3	3.7	5.1	1.9	2.0	0.7
4	0.6	4.0	5.7	2.1	2.2	0.9
5	0.9	4.2	5.9	2.4	2.5	1.1
6	1.2	4.6	6.1	2.5	2.7	1.3
7	1.5	4.8	6.3	2.6	2.9	1.6
8	1.8	5.1	6.5	2.7	3.1	1.7
9	2.1	5.4	6.6	2.8	3.4	1.9
10	2.4	5.7	6.8	2.9	3.5	2.0
11	2.7	5.9	6.9	3.1	3.8	2.2
12	3	6.0	7.0	3.2	4.1	2.3

Analytical equation that describe molybdenum content from soil correlated with molybdenum chloride concentration is $y = 0.021\ x^2 + 2.2797x + 31.045$ where correlation coefficient of *Pisum sativum* plants height for the 12 studied cases has the value $R^2 = 0.9896$. Dependence of molybdenum content by molybdenum chloride concentration in *Pisum sativum* plant parts and organs can be seen from the mathematical relations: $y = -0.1981\ x^2 + 4.9488x + 38.477$ where correlation coefficient has value $R^2 = 0.9924$ (root); $y = -\ 0.046x^2 + 2.024x + 14$ where correlation coefficient has value $R^2 = 0.9905$ (stem); $y = -\ 0.0564x^2 + 2.6429x - 0.4545$ where correlation coefficient has value $R^2 = 0.9977$ (flower).

In soil normal quantity is 2 ppm (after Romanian Lows, nr 303, 6. 11. 1997), and soil used for experiment was from Didactical Station of Timisoara having a molybdenum content of 3.5 ppm. At the end of experiment molybdenum quantity from vegetation vessels soil where grow plants treated with a solution of 3 ppm Mo^{2+} is 6.0 ppm.

From graphical representation Fig. 1 can be seen that molybdenum concentration increment in wetting solution comes to increasing quantity of heavy metal from *Pisum sativum* plant roots increase from 4.3 ppm $Mo^{6+,}$ at watering plants to 7 ppm $Mo^{6+,}$ determinate value for plants treated with solution of 3 ppm Mo^{6+}. Molybdenum content from pea stems had values between 1.6 ppm Mo^{6+} at wetness plant and 3.2 ppm Mo^{6+}, at plants treated with solutions by 3 ppm Mo^{6+} concentration. At pea leaves can be seen molybdenum increasing quantity in limits 1.2 ppm Mo^{6+} 4.1 ppm Mo^{6+}, meanwhile wetting solution concentration is increasing from 0.1 ppm Mo^{6+} at 3 ppm Mo^{6+}, the same variation can be seen at pea flowers accumulated molybdenum in quantities between 0.5 ppm to witness plant and 2.3 ppm at plants treated with solution by concentration of 3 ppm Mo^{6+}.

Table 3.

Iron, copper, calcium, potassium, sodium and phosphorus content from *Pisum sativum* plants

Nr. crt.	Molybdenum concentration from solution (ppm)	Other chemical elements content (mg%)					
		Fe	Cu	Ca	K	Na	P
1	0	5	37	10	120	1	50
2	0.1	4.5	36	9	114	0.9	51
3	0.3	4	34	8	105	0.7	53
4	0.6	3.8	34	8	98	0.7	54
5	0.9	3.7	31	7	95	0.7	55
6	1.2	3.6	31	6	93	0.5	57
7	1.5	3.5	29	6	91	0.5	57
8	1.8	3.4	26	5	87	0.5	58
9	2.1	3.1	24	5	85	0.4	59
10	2.4	2.7	23	3	84	0.2	60
11	2.7	2.5	22	3	83	0.2	62
12	3	2.3	22	2	81	0.1	62

In Table 3 and figures 1 and 2 can be seen pea plants content in iron, copper, calcium, potassium and sodium, modified by molybdenum concentration increment. It can be seen that meanwhile molybdenum ions concentration

increased, iron quantity decreased from 5 mg at witness to 2.3 mg at plants treated with solution of concentration 3 ppm Mo^{6+}. The same happens with sodium which decreased from 1 mg to 0.1 mg. Molybdenum in wetting solution had a positive influence on pea plants development.

This thing can be seen in graphical representation 3, phosphorus quantity from plants increase proportionally with increasing molybdenum quantity from 50 mg to 62 mg. Instead copper quantity decreased from 37 mg at witness sample to 22 mg at plants treated with concentrated molybdenum chloride.

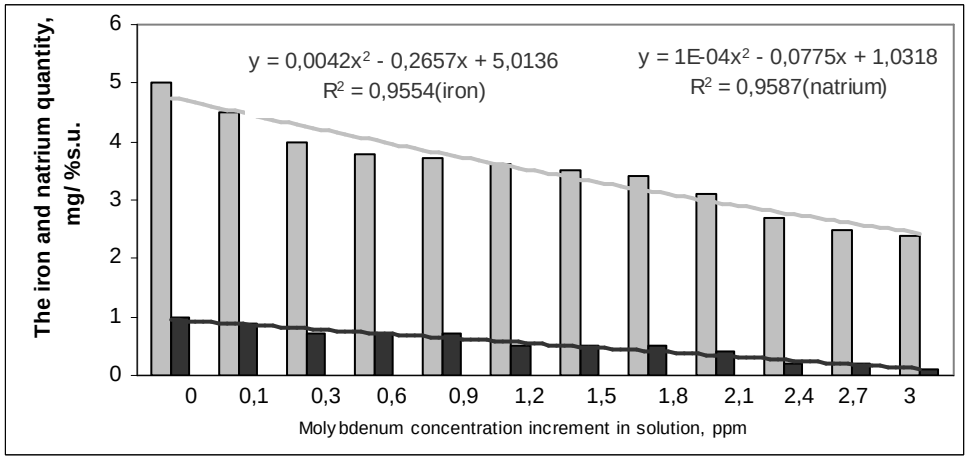

Figure 1. Graph representation of iron and sodium quantity in *Pisum sativum* plants in correlation with molybdenum concentration increment in solution

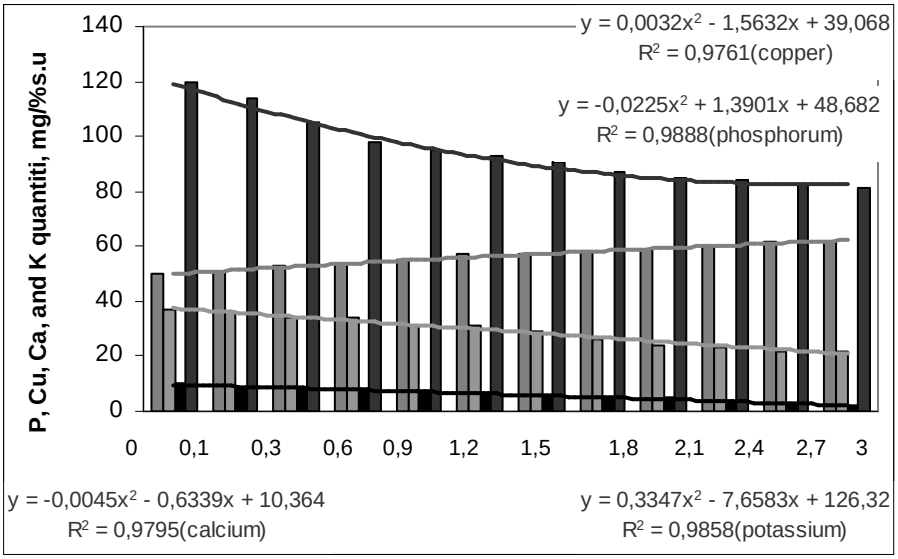

Figure 2. Graph representation of P, Cu, Ca and K quantity from *Pisum sativum* plants in correlation of molybdenum concentration increment in solution

The same calcium quantity decreased from 10 mg to 2 mg and potassium quantity decreased from 120 mg at witness sample to 81 mg in plants treated with a solution by 3 ppm Mo^{6+} concentration. These quantities were reported to 100 g dry substance.

Conclusions

Plants can assimilate big quantities of molybdenum without being affected, because accumulates molybdenum in vacuoles in safe forms. In vegetable roots molybdenum content is higher than the rest of plant because of the nitroreductase and nitrogenase activity. The biggest quantity of molybdenum from aerial part can be seen in seeds; in leaves and stems being much more reduced concentrations.

Vegetal evaluation method of soil quality after adding molybdenum had the following results: *Pisum sativum* plants treated with different molybdenum concentrations proved to be vegetal indicators accumulating this metal, without exterior important changes and chemical changes. Molybdenum has a positive effect for plants development (in all cases treated plants were much more biggest). Soil quality wasn't influenced by molybdenum because roots length increased. Molybdenum ions accumulated proportional with concentration increase in wetting solution in roots, stems, leaves and flowers in plants experimental growing but remains in vessel soil. Plant roots in all cases had superior contents of heavy metal comparing with aerial parts of plant. When molybdenum accumulated in pea plants others chemical elements decreased, but in normal limits. The exception is phosphorus. Molybdenum concentration varieties by plant regions. Vegetable roots where activity of nitroreductase and nitrogenase is intense, molybdenum content is higher than in aerial part of plant. The biggest quantity of molybdenum is in aerial part, in leaves, meanwhile in flowers and steams the concentrations are reduced. Molybdenum content from seeds cans varieties from one soil to another. Knowing this, on the soils with molybdenum deficit, the level of the metal, from seeds can be a prognoses criterion of a possible molybdenum deficit.

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