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IMPACT ASSESSMENT REGARDING POLLUTION WITH NUTRIENTS OF WATER RESOURCES DUE TO THE USE OF FERTILIZERS WITH MICROBIAL BIOMASS

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Abstract

A way of valorise the solid by-products resulting from industrial biosyntheses is to use them as such to fertilize agricultural soils or to obtain new types of fertilizers. Two types of fertilizers obtained with spent microbial biomass were evaluated regarding the impact generated on the waters as a result of potential nutrient loss processes through washing throughout rainwater. The tests were carried out in the lab, in glass columns filled with a mixture of red-brown soil, sand, and fertilizers, the last one added in quantities corresponding to a fertilization norm of 200 kg SA/ha. For each experimental variant, an average pluviometry flow rate of 14 L/m² was simulated; in each column, water was added at intervals of 1; 4; 7, and 15 days. The nutrients lost by leaching were assessed after each interval. The obtained results showed that fertilizers formulated with microbial biomass lose (77.9-85.11)% P_2O_5 and (76.2-90)% K_2O in 24 h, compared to classic fertilizers, which lose 75% P_2O_5 through leaching, respectively 86.5% K_2O. Because the behaviour of fertilizers formulated with microbial biomass is similar to that of complex fertilizers, was estimated that the impact generated by the use of fertilizers with microbial biomass on the water is insignificant.

***Key words**: fertilizer, microbial biomass.*

INTRODUCTION

In the current context, biotechnological processes (white or red biotechnologies) generate large quantities of by-products (microbial biomass) that are stored in dumps (Sulivan et al., 2017; Halter et al., 2020). These deposits often cause environmental problems, because they block large areas of land, from where the wind and rainfall can transport it in air, soil, water or groundwater polluting all environmental factors (Gowariker et al., 2009; Jayanta et al., 2028) Over time, the valorisation of spent microbial biomass has been made in three main directions: 1) obtaining absorbents used to reduce the load of metal cations present in industrial wastewater (Radu et al., 2006; Radu et al., 2007a; Dima et al., 2006; Migahed et al., 2017; Chwastowski, et al., 2023); 2) conditioning them in the form of organic fertilisers, after thermal or chemical inactivation (Andersen et al., 2001; Halter et al., 2020; Stikane et al., 2022; Stikane et al., 2023; Vurukonda et al., 2024) or using them in the formulation of fertiliser type NP/NK/NPK/NPK with microelements (Radu & Meghea, 2007b; Radu et al., 2020) and 3) combustion under controlled conditions to obtain the biochar (Meng et al., 2023). Actual environmental legislation together with the reduction of available land for the storage of spent microbial biomass, has forced large biotech companies to look for profitable ways to capitalize on this waste. A sustainable way of valorising the by-products resulting from biotechnological processes is to use them to obtain new fertilizers, which can be used in agriculture (Radu et al., 2006; Meghea et al., 2007; Radu et al., 2020). The products obtained with microbial biomass combine two qualities: they have fertilization properties, thanks to the nutrients in the biomass (Arrieche-Luna, 2010; Černý et al., 2023), and

they act as precursors for humic compounds, which improve the agrochemical properties of the soil (Azizah et al., 2023; Wang et al., 2023).
The main purpose of the conducted studies was to evaluate the impact generated on water sources (surface water; underground water) as a result of the use of NP/NPK/NK type fertilizers formulated with microbial biomass functionalized with microelements.
The studied fertilizers were obtained in the lab according to the methodology presented by Radu and colab. (Radu et al., 2007b; Meghea et al., 2007; Radu et al., 2020).

MATERIALS AND METHODS

To evaluate the impact on water resources generated by fertilizers with microbial biomass, the leaching tests were performed with fertilizers formulated with two spent microbial biomass types: *Penicillium* sp. (PB) and *Streptomyces* sp., (SB) resulting from the current pharma bioprocesses. Elemental composition of the two types of microbial biomass is shown in the Table 1.

Table 1. Elemental composition of microbial biomass

Element	C %	Ca %	Mg %	N %	P_2O_5 %	Co µg/g	Cu µg/g	Fe µg/g	Mn µg/g	Zn µg/g
	Concentration									
Biomass 1 (SB)	36.8	3.73	0.013	6.24	0.11	30.47	1.71	938.3	24.3	26.6
Biomass 2 (PB)	30.3	0.32	0.001	1.84	1.44	1.73	28.74	705.2	5.11	52.3

The two types of microbial biomass were tested alone (PB and SB) or as a fertilizers type NPK (9:8:4; 11:22:5; 13:13:7; 17:41:9), obtained with biomass of SB. The tests were carried out in glass columns, in which were introduced a mixture of red-brown soil and sand (mass ratio: 1:1). Fertilizers were introduced in columns in amounts corresponding to the fertilization norm of 200 kg SA/ha, after which an average pluviometry flow rate of 14 L/m^2 was simulated at each watering.
The water was added in columns used in the experiment at four intervals: 24h; 96h; 168h and 360h. Nutrients lost by leaching were made from the effluents obtained in the collector vessel, after each interval.
Each experiment was performed in three times, and results were presented as average value, with corresponding standard deviation. The loss of microelements and macroelements by washing were measured after removing of organic compounds from aqueous effluents (Radu et al., 2020), using an ICP AES spectrometer type VARIAN Liberty 110.

RESULTS AND DISCUSSIONS

Results obtained after measurements done after 24 h show that in the case of SB, this generally retains much better their constituents, in comparison to the PB. In the case of main (or major) macronutrients, results obtained show the following:
- for magnesium: the biomass derived from SB loses 4.6% of this in comparison with the PB, which loses 25.4% (Figure 1 and Figure 2);
- in the case of calcium, the SB loses 19.5% Ca while the PB loses 48.8% Ca (Figure 1 and Figure 2);
- regarding P_2O_5, the SB loses 1.1% in comparison with the PB, which loses 2.9%. (Figure 1 and Figure 2).

Potassium (expressed as K_2O), from SB, loses 30.4% in comparison with the PB, which loses 66.7% (Figure 1 and Figure 2).
Regarding the macroelements lost by leaching, it is found that the PB loses 2 times more nutrients in comparison with SB.
In the terms of microelements, the amount lost by leaching is much higher for PB except the Fe, where the SB loses 1.7%, more than PB, which loses only 0.5% (Figure 3 and Figure 4).
In the case of the PB, this loses its microelements in greater quantities, respectively 47.6% Co, 34.3% Cu, 46.3% Mn, 100% Mo, and 3.1% Zn, in comparison with the SB (Figure 3 and Figure 4), which loses microelements in smaller quantities: 28.7% Co, 7.1% Cu, 28.4% Mn, 100% Mo, 0.6% Zn, except Mo, for which they both subproducts lost Mo in the same quantities (100%).
Keeping into account the concentration levels of macroelements and microelements in the effluents tested, we consider that the environmental risk due to lost macroelements and microelements leached from both microbial biomasses is insignificant (Table 2), because the concentration levels in each effluent are smaller than intervention limits imposed by legislation in force (NTPA 011).

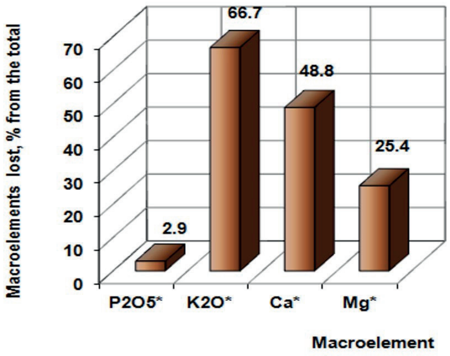

Figure 1. Macronutrients lost by leaching from the PB after 24 hours

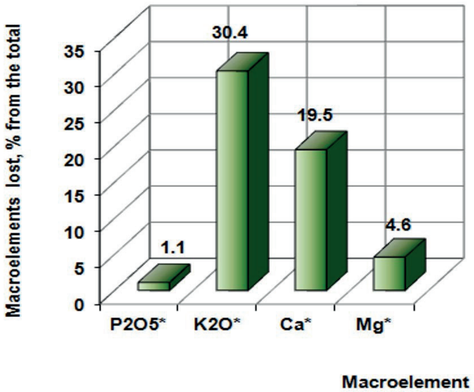

Figure 2. Macronutrients lost by leaching from the SB after 24 hours

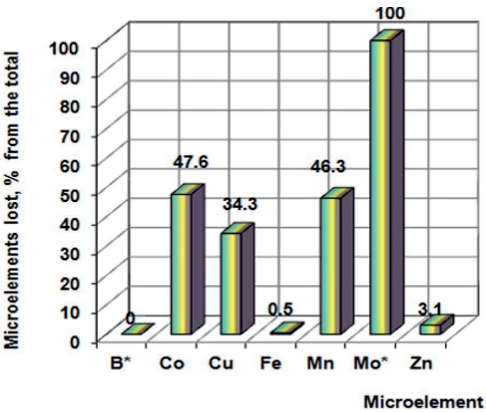

Figure 3. Micronutrients lost through leaching from microbial biomass of PB after 24 h

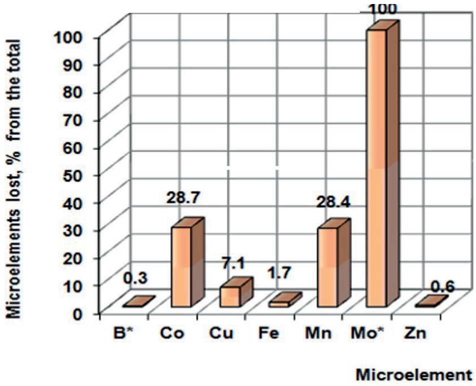

Figure 4. Micronutrients lost through leaching from microbial biomass of SB after 24 h

Table 2. Risk assessment regarding water pollution with elements from microbial biomass

Element (ppm)	Maximum permissible concentration (admissible level, ppm)		Elements lost due to leaching from SB	Elements lost due to leaching from PB	Environmental impact
	Household receivers (sewage)	Natural receptors			
Ca	-	300	0.4296	0.0332	
Mg	-	100	0.0868	0.0053	
P	5	1(2)	0.0280	0.0545	
Cu	0.2	0.1	0.0998	0.0657	
Co	-	1	0.0339	0.0136	INSIGNIFICANT
Fe	-	5	0.0479	0.0017	
Zn	1	0.5	0.0046	0.0041	
Mn	2	1	0.0445	0.0145	
Mo	-	0.1	0.0200	0.0038	

Regarding the leachability in time of fertilizers with microbial biomass, the results obtained until 360 hours with the four types of fertilizers studied, (two fertilizers formulated with microbial biomass respectively A: NPK 9:8:4 and B: NPK 11:22:5 and two types are conventional fertilizers C: NPK 11:22:5 and D: NPK 17:41:9) showed the following:

- in the case of P_2O_5, the fertilizers formulated with microbial biomass lose about (77.9-85.11)% P_2O_5 on the first day (24 h) (Figure 5), in comparison with classical fertilizers, where the degree of loss is below 75%;
- after 360 h, the P_2O_5 loss by leaching is in the proportion of (96-98)%;

- the potassium loss (expressed as K_2O) is situated in the range of (76.2-90)% in 24 h (Figure 6), in the case of fertilizers formulated with microbial biomass, while for the classic fertilizers, the K_2O loss is situated below 86.5%;

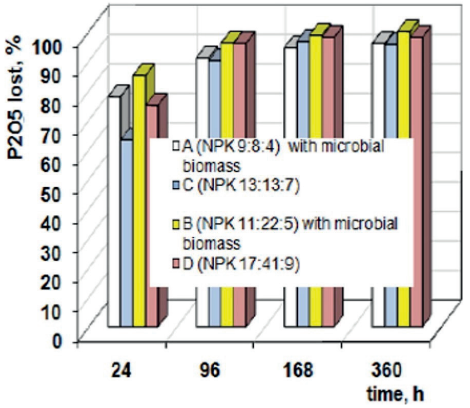

Figure 5. P_2O_5 loss through leaching in time

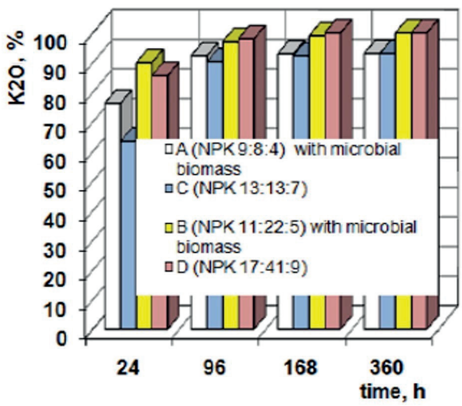

Figure 6. K_2O loss through leaching in time

- at 360 h, a proportion of (93-100%) from K_2O is lost by leaching in the case of fertilizers with microbial biomass, and respectively 93-100% is lost by leaching in the case of classic NPK fertilizers. Regarding the Ca element: this is loosed totally by leaching at 24 h for all types of fertilizers that contain microbial biomass (Figure 7). In the case of Mg, at 24 h, this is lost in the proportion of 63% for mixed fertilizers (Figure 8) which contain microbial biomass (variant A), and in the proportion of 42% for classic NPK.

At 96 h, the losses of Ca and Mg are almost total 99-100%. Taking into account the fact that the lost nutrients from both types of fertilizers (mixed fertilizers formulated with microbial biomass and classic fertilizers type NPK) have similar values, we consider that the environmental impact generated by leaching nutrients from fertilizers with microbial biomass in waters is insignificant.

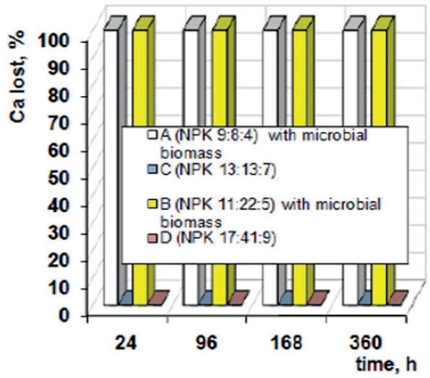

Figure 7. Ca loss through leaching in time

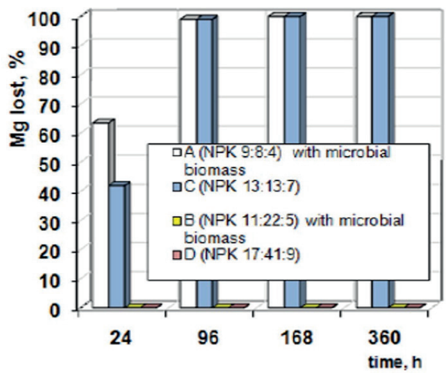

Figure 8. Mg loss through leaching in time

Similar results have been reported in other studies regarding the environmental impact of using microbial biomass generated as by-products from biotechnological processes in agriculture. Thus, Andersen and colab. (Andersen et al., 2001) have demonstrated through their studies carried out in Denmark on soils fertilized for 7 years with inactive spent biomass, derived from genetically modified microorganisms used in pharma biosyntheses, that this fertilization technique does not affect the soil microbiome. The molecular analyses performed on to microorganisms isolated from these soils did not reveal the existence of genes/gene fragments similar to those existing in the microorganisms from the inactive spent microbial biomass (Andersen et al., 2001). Similar results were also obtained in the research carried out in the USA by Sullivan et al., on the soils cultivated with *Zea mays*, fertilized with inactivated spent microbial biomass (Sullivan et al., 2017), in the sense that in the microorganisms present in the soil fertilized in this way, were not highlighted gene transfer from the spent inactivated biomass used as fertilizer. Instead, in all cases where biomass-based products were used for fertilization, positive effects were reported regarding the growth and development of crop plants, the yields obtained per hectare in the case of the use of these bioproducts being similar to those obtained by applying the fertilizers obtained throughout chemical synthesis (urea) (Sullivan et al., 2017; Wang et al., 2023). Moreover, Halter and colab. (Halter et al., 2020) the tests carried out in Tennessee, USA, found that at a fertilization rate of 7.5 t/ha with inactive biomass, the soil microbiota is characterized by copiotrophic microorganisms (microorganisms specific to soil sites rich in nutrients).

In addition, under the influence of microbial biomass, as well as vegetal biomass, the soil microbiome and microbial activity improve (Nakhro et al., 2010; Wang et al., 2023); the content of organic C and N in the soil increases; the physical-chemical parameters of the soil (pH; respiration; enzymatic activity) is improved and the agrochemical quality of the soil increases (Meng et al., 2023; Azizah et al., 2023). According to the European Union, the transition from an oil-based economy to a biomass-based economy (Bioeconomy) will represent a characteristic of the 21st century due to the exhaustion of natural resources. The transition will bring back to the fore microbial biotechnologies and generate the huge amounts of biomass that must be capitalized (Stikane et al., 2023). An advantageous option is the re-use of these bioresources in sustainable agriculture to obtain fertilizers (Radu et al., 2020; Stikane et al., 2023) or biochar (Manolikaki & Diamadopoulos, 2020). These options of valorisation will supply nutrients for crops (Manolikaki et al., 2020; Radu et al., 2020; Vukuonda et al., 2024), will improve the agrochemical properties of the soil and most importantly aspect, will affect insignificantly water resources. In this regard, innovative companies in the field of biotechnologies have made more industrial processing scenarios, with spent microbial biomass as a raw material, with concepts of economic sustainability and environmental protection (Stikane et al., 2023; He-Lambert et al., 2019).

CONCLUSIONS

In terms of resistance at leachability for the fertilizers with microbial biomass, is found that the materials which contain biomass of *Streptomyces sp.* are more resistant to loss by leaching in comparison with fertilizers that contain the biomass of *Penicillium* sp. For this reason, only the Streptomyces biomass was used as raw materials for obtaining fertilizers. The leachability tests conducted at the lab scale indicate that the leaching behaviour of fertilizer with microbial biomass of Streptomyces sp. is comparable to that of complex NPK fertilizers. Consequently, it is estimated that the impact on water resources from the use of these new types of fertilizers is insignificant.

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