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# EFFECT OF THE BIOSTIMULANT KELPAK SL ON THE CONTENT OF SOME MICROELEMENTS IN TWO GRASS SPECIES

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**Abstract**

Seaweed extracts have been recently introduced to crop growing, particularly to sustainable agriculture, in many countries worldwide. However, our knowledge of the action produced by Kelpak is only fragmentary as it is influenced by a number of factors, for example crop plant species and extract application schedule. Our objective was to determine the effect of Kelpak SL on the content of selected microelements in two grass species. A field experiment was arranged in a randomized sub-block design (split-split-plot) with three replicates. It was conducted at the Experimental Unit of the University of Natural Sciences and Humanities in Siedlce (Poland) and started in late April each year. The following factors were examined: pure stands of two grass species *Dactylis glomerata* L. (cv. Amila) and *Festulolium braunii* (K.Richt.) A. Camus (cv. Felopa) grown in a monoculture, a biostimulant distributed under the trade name Kelpak SL, applied at 2 $dm^3$ $ha^{-1}$ (no biostimulant in the control treatment), and nitrogen applied at 50, 100, 150 kg $ha^{-1}$ (no nitrogen in the control). During the experiment, grass was cut three times a year. The plant material was subjected to chemical analyses to assess dry matter (by determining the moisture content), zinc, copper, iron and manganese. The application of Kelpak significantly increased the Zn, Cu, Fe and Mn content in the grass species tested, regardless of the remaining factors. The grass species did not differ significantly in their content of Zn, Cu, Fe and Mn. The concentrations of the microelements in both species were significantly affected by a dose of nitrogen. At higher nitrogen doses, the concentrations of Zn, Cu and Fe were lower, unlike the content of Mn, which increased. The Fe:Mn ratio in the dry matter of both grasses was 2.79, which indicates some manganese deficiency.

**Keywords:** *Dactylis glomerata, Festulolium brauni*, seaweed extract, Zn, Cu, Fe, Mn.

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## INTRODUCTION

The intensification of agricultural production, which involves the cultivation of crops with high nutrient and fertiliser needs, contributes to some considerable removal of microelements from soil. Their depletion can be counteracted through the soil application of farmyard manure, sewge sludge (Kalembasa, Godlewska 2010) and, to a lesser degree, mineral fertilisers (Kaniuczak et al. 2009). Another solution recently applied globally in crop growing, particularly in sustainable agriculture, is the use of seaweed extracts. They can be used as biostimulants and liquid fertilisers owing to their content of phytohormones, such as auxins, cytokinins and gibberellins (Durand et al. 2003, Strik et al. 2004), macroelements – Ca, K and P and microelements – Fe, Cu, Zn, B, Mn, Co and Mo (Khan et al. 2009, Craigie 2011). It has been reported in literature that an application of algal extracts increases the soil content of trace elements (Dobrzański et al. 2008), enhances plant yield and quality, improves plant resistance to pests and pathogens (Verkleij 1992) and to drought and soil salinization. Kelpak SL is a product based on seaweed extract, obtained from sea (brown) algae *Ecklonia Maxima*, which are harvested off the shore of South Africa. It contains auxins (11 mg $l^{-1}$) and cytokinins (0.03 mg $l^{-1}$). Research has confirmed that the product has a positive effect on crop plants. However, our knowledge of the action of Kelpak is only fragmentary as it is influenced by a number of factors, for example crop plant species and extract application time (Matysiak et al. 2012). The objective of the study was to determine the effect of Kelpak SL on the content of selected microelements in two grass species.

## MATERIAL AND METHODS

A field experiment was arranged in a randomized sub-block design (split-split-plot) with three replicates. It was set up in late April 2009, at the Experimental Unit of the University of Natural Sciences and Humanities in Siedlce (Poland). Each plot covered 10 $m^2$. The soil belonged to Hortic Anthrosol soils (WRB). Soil chemical composition analyses were carried out at the National Chemical and Agricultural Station in Warsaw. The soil had neutral pH, was rich in copper, zinc, available phosphorus and magnesium, but moderately rich in manganese, total nitrogen and available potassium (Table 1). The following factors were examined:

– pure stands of grass species grown in a monoculture;
  - *Dactylis glomerata* L. cv. Amila,
  - *Festulolium braunii* (K.Richt.) A. Camus, cv. Felopa;
– biostimulant distributed under the brand name Kelpak SL, applied at 2 $dm^3$ $ha^{-1}$ and no biostimulant in the control treatment;
– nitrogen applied at 50, 100, 150 kg $ha^{-1}$, and 0 kg $ha^{-1}$ in the control.

Table 1

Chemical composition of soil

| pH in KCl | Content of assimilable components (mg $kg^{-1}$ of soil) | | | (g $kg^{-1}$ d.m. soil) | (mg $kg^{-1}$ d.m. soil) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | P | K | Mg | N - total | $N\text{-}NO_3$ | $N\text{-}NH_4$ | Cu | Zn | Mn |
| 7.2 | 392 | 160 | 84 | 1.8 | 10.10 | 7.47 | 28.7 | 150.7 | 162.0 |

During the experiment (2010-2012), the grass cutting regime consisted of three harvests a year. Ammonium nitrate was applied three times a year. The total nitrogen amount was split into three equal doses, which were applied before each cutting. The P and K fertilisation was applied to all the plots. Phosphorus was applied once as triple superphosphate at a dose of 17.4 kg $ha^{-1}$ P in the spring. The amount of potassium (132.8 kg $ha^{-1}$ K) was split into three equal doses and applied as 60% potash salt prior to each cutting. The biostimulant Kelpak was sprayed as an aqueous solution, in a dose of 2 $dm^3$ of the preparation diluted in water to 400 $dm^3$ per hectare. The spraying was performed before each grass harvest; the first application was three weeks before the first cut, the second one was scheduled two weeks after the first cut, and the last treatment was performed three weeks after the second harvest.

During each harvest, 0.5 kg samples of grass green matter were taken from each plot to carry out chemical analyses. The samples were left to dry in a ventilated room. The air-dried matter was shredded and ground, after which the plant material was subjected to chemical analysis to determine dry matter (by determining the moisture content), zinc, copper, iron and manganese. All the microelements were determined by the AAS method. The results were used to calculate the Fe:Mn ion ratio in grass dry mater for each experimental combination.

Statistical analysis was conducted using the program Statistica StatSoft, Inc. (2011). Statistica (data analysis software system), version 10 (www.statsoft.com). Significance of differences between means was verified with the Tukey's test at the significance level of $\alpha \leq 0.05$.

The weather conditions were changeable during the study period (Table 2). The average air temperatures and precipitation sums in all the growing seasons were higher than the long-term means but the precipitation was very unevenly distributed. In 2010 and 2011, the rainfall was 115.3 and 80.5 mm higher, respectively, than the long-term means. It is worth noting that in July 2010 the precipitation was 4.5-times higher than the long-term mean for July, and it corresponded to 48% of the rainfall in the whole growing season. By contrast, a severe rainfall deficit was recorded in April 2010 and in September 2011.

Table 2

Meteorological conditions in the growing season 2010-2012, recorded at the meteorological station in Siedlce

| Year | Means air temperatures (°C) | | | | | | Means in growing season |
|---|---|---|---|---|---|---|---|
| | Apr | May | June | July | Aug | Sept | |
| 2010 | 8.9 | 14.0 | 17.4 | 21.6 | 19.8 | 11.8 | 15.6 |
| 2011 | 9.8 | 13.4 | 18.1 | 18.2 | 18.1 | 14.4 | 15.3 |
| 2012 | 9.0 | 14.5 | 16.4 | 20.4 | 18.0 | 14.2 | 15.4 |
| Means of many years (2002-2012) | 7.7 | 10.0 | 16.1 | 19.3 | 18.0 | 13.0 | 14.0 |
| Year | monthly precipitations (mm) | | | | | | sum in growing season |
| | Apr | May | June | July | Aug | Sept | |
| 2010 | 10.7 | 93.2 | 62.6 | 77.0 | 106.3 | 109.9 | 459.7 |
| 2011 | 38.1 | 55.6 | 44.3 | 204.2 | 55.4 | 26.6 | 424.2 |
| 2012 | 40.3 | 59.7 | 118.7 | 41.4 | 64.1 | 30.8 | 355.0 |
| Means of many years (2002-2012) | 52.3 | 50.0 | 68.2 | 45.7 | 66.8 | 60.7 | 343.7 |

## RESULTS AND DISCUSSION

The Zn and Cu concentrations in meadow grass that are optimal for the nutrition of ruminants equal 30-50 Zn mg $kg^{-1}$ and around 7 Cu mg $kg^{-1}$ (Wyłupek 2003). The average content of the elements in the plants tested in our experiment was slightly lower than the above values. Regardless of the nitrogen fertilisation, grass species, cuts and study years, an application of Kelpak significantly increased the concentrations of Zn (by 10.3%) and Cu (9.1%) in grass plants (Table 3). Zodape et al. (2009) applied *Kappaphycus alverezii* extract and obtained a 4.9% increase in the wheat grain zinc content. The growth regulator tested in the present study significantly increased the Zn content in grass grown at all the N doses applied. Similar relationships were detected for Cu, although when nitrogen was used at 150 kg $ha^{-1}$, Kelpak increased the N content in grasses and the differences were insignificant.

Many authors ( Matysiak et al. 2012, Godlewska, Ciepiela 2013) have found that the efficiency of a biostimulant depends, among others, on plant species. Nevertheless, the seaweed extract tested in the present study did not affect zinc or copper in the dry matter of the grasses. It should be noted that the differences in the concentrations of the elements studied in *Dactylis glomerata* and *Festulolium braunii* were not significant either, irrespective of the application of Kelpak.

Table 3

Effect of the biostimulant Kelpak SL on the dry matter of grasses content of zinc and copper by N dose, grass species, cut and study year

| Factor | | Content of Zn ( mg $kg^{-1}$ d.m.) | | | Content of Cu (mg $kg^{-1}$ d.m.) | | |
|---|---|---|---|---|---|---|---|
| | | treatment | | mean | treatment | | mean |
| | | I | II | | I | II | |
| N dose (kg $ha^{-1}$) | 0 | 26.77*a* | 30.73*b* | 28.75*A* | 7.03*a* | 7.92*b* | 7.48*A* |
| | 50 | 24.48*a* | 27.41*b* | 25.94*B* | 6.53*a* | 7.19*b* | 6.86*B* |
| | 100 | 23.75*a* | 25.87*b* | 24.80*BC* | 6.04*a* | 6.68*b* | 6.35*C* |
| | 150 | 22.15*a* | 24.27*b* | 23.21*CD* | 5.95*a* | 6.32*a* | 6.13*C* |
| Species of grass | *Dactylis glomerata* | 24.28*a* | 27.15*b* | 25.71*A* | 6.41*a* | 7.04*b* | 6.72*A* |
| | *Festulolium braunii* | 24.30*a* | 27.00*b* | 25.64*A* | 6.40*a* | 7.02*b* | 6.69*A* |
| Cut | 1 | 27.72*a* | 30.56*b* | 29.14*A* | 5.27*a* | 5.83*b* | 5.55*A* |
| | 2 | 21.40*a* | 23.92*b* | 22.66*C* | 6.53*a* | 7.19*b* | 6.86*B* |
| | 3 | 23.75*a* | 26.73*b* | 25.24*B* | 7.36*a* | 8.07*b* | 7.71*C* |
| Year | 2010 | 26.44*a* | 29.20*b* | 27.81*A* | 6.62*a* | 7.22*b* | 6.92*A* |
| | 2011 | 19.76*a* | 21.96*b* | 20.86*B* | 5.99*a* | 6.63*b* | 6.31*B* |
| | 2012 | 26.67*a* | 30.04*b* | 28.36*A* | 6.56*a* | 7.23*b* | 6.89*A* |
| Mean | | 24.29*a* | 27.07*b* | 25.68 | 6.39*a* | 7.03*b* | 6.71 |

I – without biostimulant ( control); II – with biostimulant Kelpak SL (2 $dm^3$ $ha^{-1}$);
Different letters in the same row indicate significant differences;
Values in columns for individual factors assigned different capital letters differ significantly.

The grass content of Zn and Cu was significantly affected by nitrogen doses. Increasing nitrogen amounts reduced the grass content of both microelements, which has been confirmed by Kleiber and Komosa (2011). Vuckovic et al. (2005) demonstrated that higher nitrogen doses reduced the zinc content in grasses but copper accumulation was increased.

Substantial variation detected in the plant content of microelements may also be caused by the external conditions during the growing season (Kaniuczak et al. 2009). In our experiment, the least Zn was determined in grass harvested at second cut and the highest Zn content was determined in grass from the first cut, with the differences being statistically significant. By contrast, the copper concentration in the plants tested increased at the successive harvests, and the differences were also statistically significant.

Considering the years of the experiment, the significantly lowest Zn and Cu concentrations were detected in grass harvested in the second year.

The levels of iron and manganese in feed should be 50-200 mg $kg^{-1}$ and 50-100 mg $kg^{-1}$, respectively. In our reearch, the iron content was optimal in all the plant samples, whereas the manganese content was slightly insufficient (Table 4). This was probably due to the fact that Mn availability is hi-

Table 4

Effect of the biostimulant Kelpak SL on the dry matter of grasses content of iron and manganese by N dose, grass species, cut and study year

| Factor | | Content of Fe ( mg kg$^{-1}$ d.m.) | | | Content of Mn (mg kg$^{-1}$ d.m.) | | |
|---|---|---|---|---|---|---|---|
| | | Treatment | | Mean | Treatment | | Mean |
| | | I | II | | I | II | |
| N dose (kg ha$^{-1}$) | 0 | 139.1*a* | 162.3*b* | 150.7*A* | 42.78*a* | 46.37*b* | 44.58*A* |
| | 50 | 128.5*a* | 143.0*b* | 135.8*B* | 45.77*a* | 48.61*b* | 47.19*B* |
| | 100 | 123.2*a* | 135.5*b* | 129.4*C* | 48.07*a* | 50.99*b* | 49.53*C* |
| | 150 | 116.6*a* | 125.6*b* | 121.1*D* | 50.35*a* | 55.03*b* | 52.69*D* |
| Species of grass | *Dactylis glomerata* | 126.5*a* | 139.8*b* | 133.1*A* | 46.80*a* | 50.29*b* | 48.54*A* |
| | *Festulolium braunii* | 127.3*a* | 143.5*b* | 135.4*A* | 46.69*a* | 50.21*b* | 48.45*A* |
| Cut | 1 | 122.1*a* | 137.3*b* | 129.7*A* | 45.66*a* | 48.93*b* | 47.29*A* |
| | 2 | 127.1*a* | 140.5*b* | 133.8*A* | 45.94*a* | 49.45*b* | 47.70*A* |
| | 3 | 131.4*a* | 147.1*b* | 139.3*B* | 48.64*a* | 52.37*b* | 50.51*B* |
| Year | 2010 | 129.5*a* | 144.4*b* | 136.9*A* | 45.87*a* | 49.34*b* | 47.60*A* |
| | 2011 | 123.0*a* | 137.6*b* | 130.3*B* | 47.61*a* | 51.30*b* | 49.45*B* |
| | 2012 | 128.1*a* | 143.0*b* | 135.5*AC* | 46.75*a* | 50.11*b* | 48.43*AB* |
| Mean | | 126.9*a* | 141.6*b* | 134.23 | 46.74*a* | 50.25*b* | 48.95 |

Key under Table 3

gher in acid and alkaline soils, but the soil under the experimental plants was neutral.

The application of the biostimulant Kelpak, regardless of the remaining factors, significantly increased the Fe and Mn content in the plants tested. Our statistical analysis demonstrated a significant interaction of the extract with nitrogen fertilisation. At each nitrogen level, Kelpak significantly increased the Fe and Mn content of the grass species. The concentrations of Fe and Mn in both the grasses did not change significantly, irrespective of the remaining experimental factors. However, following an application of Kelpak, the dry matter content of these microelements significantly increased in both *Dactylis glomerata* and *Festulolium braunii*.

Our analysis of the data from each cut and year revealed a significant increase in the iron and manganese content in each grass cut following an application of the algal extract. Nitrogen fertilisation significantly affected Fe and Mn in the grasses. The plant content of iron declined as the nitrogen fertiliser doses increased, in contrast to manganese. As the nitrogen doses rose, the Mn content in the grasses increased significantly. Also, Malhi et al. (1998) reported a higher manganese content in plants fertilised with nitrogen.

Comparison of the data from the three cuts demonstrated that Fe and Mn concentrations were significantly higher in the third cut. Oresnik et al.

(1999) observed different manganese concentrations in grass plants depending on the cut. In the successive years, the grass concentration of iron differed significantly, whereas the difference in the manganese content was significant between the first and second study year.

Diverse meteorological conditions, in particular fluctuations in the precipitation (Table 2), did not affect the content of Zn, Cu, Fe and Mn in the plants tested. The results cannot be discussed due to the lack of relevant literature.

According to Wierzbicka and Trawczyński (2011), the iron-to-manganese ratio in plants fed to animals should be 1.5-2.5:1. The ratio of iron and manganese in dry matter of the analyzed grasses (Table 5) averaged to 2.79.

Table 5

Effect of the biostimulant Kelpak SL on the ratio of iron and manganese in the dry matter of grasses by N dose, grass species, cut and year

| Factor | | The ratio of iron and manganese | | |
|---|---|---|---|---|
| | | treatment | | mean |
| | | I | II | |
| N dose (kg ha$^{-1}$) | 0 | 3.25*a* | 3.50*b* | 3.38*A* |
| | 50 | 2.81*a* | 2.95*b* | 2.88*B* |
| | 100 | 2.57*a* | 2.66*a* | 2.62*C* |
| | 150 | 2.32*a* | 2.28*a* | 2.30*D* |
| Species of grass | *Dactylis glomerata* | 2.73*a* | 2.81*a* | 2.77*A* |
| | *Festulolium braunii* | 2.75*a* | 2.89*b* | 2.82*A* |
| Cut | 1 | 2.70*a* | 2.84*b* | 2.77*A* |
| | 2 | 2.79*a* | 2.87*a* | 2.83*A* |
| | 3 | 2.72*a* | 2.84*b* | 2.78*A* |
| Year | 2010 | 2.84*a* | 2.96*b* | 2.90*A* |
| | 2011 | 2.61*a* | 2.71*a* | 2.66*B* |
| | 2012 | 2.76*a* | 2.88*b* | 2.82*AC* |
| Mean | | 2.74*a* | 2.85*b* | 2.79 |

Key under Table 3

When this ratio is wider than 2.5, manganese deficiency symptoms are observed and iron excess becomes harmful (Rogóż 2009). An application of the seaweed extract significantly increased the ratio regardless of the remaining factors examined in the experiment. The interaction of the growth regulator and nitrogen fertilisation was significant in two cases: when no nitrogen was applied or the N dose was 50 kg ha$^{-1}$. At 100 kg N ha$^{-1}$, the Fe:Mn ratio also increased but the differences were not significant. The effect of Kelpak on the Fe:Mn ratio was not identical in the two grass species. The ratio changed following an application of Kelpak only in *Festulolium braunii*. With respect to the cuts (two-year means), Kelpak increased the Fe:Mn ratio of grass harvested at the first and third cut. Also, the effect of the growth regulator in individual years was different, a significant influence of Kelpak on the Fe:Mn ratio in grasses being observed in 2010 and 2012.

The statistical analysis demonstrated that increasing nitrogen doses reduced the Fe:Mn ratio. Its value did not change significantly in the successive cuts, regardless of the remaining experimental factors. In contrast, significant differences between values of the Fe:Mn ratio in grasses were found between the years.

## CONCLUSIONS

1. Kelpak, a seaweed extract, significantly increased Zn, Cu, Fe and Mn in the grasses tested, regardless of the remaining factors.

2. The grass species did not differ significantly in the Zn, Cu, Fe and Mn content. The dry matter content of Fe and Mn increased following an application of the seaweed extract.

3. The concentrations of microelements in both grass species were significantly affected by nitrogen fertilisation. As the nitrogen doses increased, the concentration of Zn, Cu and Fe declined, only Mn being an exception.

4. The Fe:Mn ratio in the dry matter of grasses was 2.79, whitch indicates manganese deficiency

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