

Novel Biostimulant “Bioragi” Boosts Plant Development and Limits Trace Metal Absorption

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The world's population has exceeded eight billion, which will necessitate a tripling of food production in the next three decades to meet basic human needs. The world is now on the verge of a new “Green Revolution”. New agrobioorganic technology represents one of the ways to address famine and malnutrition by enabling sustainable food production. Additionally, it is a means of increasing both the quantity and quality of agricultural products while reducing the negative environmental impact of chemicalization, leading to significant economic, ecological, and social benefits. The elements evaluated in the study are also heavy metals and are harmful to human health. This study investigated the impact of the biostimulant “bioragi” (produced in Georgia) on sugar beet plants. The accumulation of trace metals in plant organs was studied dynamically. Observations were made on the growth, development, and sucrose content of sugar beet mass. The trace metals studied included Ti, V, Cr, Co, Ni, Cu, Zn, As, Rb, Sr, Mo, Cd, Cs, Ba, Pt, Au, Pb, and Th. Results indicate that the biostimulant bioragi reduced the absorption of trace metals by at least 18% compared to the control plants. Additionally, the mass and sucrose contents of sugar beet plants treated with bioragi increased compared to the control plants.

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INTRODUCTION

In order to ensure the food needs of our planet's growing population under the additional pressure of climatic changes and political crises, both socio-economic and technological challenges have to be met. A significant portion of the world's population has no access to adequate food, and it is stated that approximately 830 million people suffer from chronic famine (FAO, 2022). The overarching technological challenge is to increase productivity in a sustainable way (Şevik *et al.* 2024). Farming practices must be developed that are safe and do not degrade our environment (Lichtfouse *et al.* 2009) or threaten biodiversity (Tsiafouli *et al.* 2015). It is particularly urgent to preserve the full functionality of soils and avoid soil degradation (Kopittke *et al.* 2019).

A fundamental cause of the ecological crisis is the heavy reliance on fertilizers and pesticides in present-day agricultural practices, which significantly contribute to environmental pollution (Shcherbak *et al.* 2014; Naher *et al.* 2021). This pollution can lead to a reduction of biodiversity (Knapp *et al.* 2018), and to various human health issues (Nicolopoulou-Stamati *et al.* 2016). Specifically, the increased application of pesticides

has been associated with a rise in the number of children with mental disabilities (Yang *et al.* 2023; Yiğit *et al.* 2024). Moreover, the use of chemicals does not guarantee the high quality or safety of agricultural products (Tudi *et al.* 2021). Unfortunately, complete elimination of chemical inputs in agriculture is currently unfeasible; however, it is possible to mitigate the negative impacts of chemicalization on the environment by minimizing their use (Cai *et al.* 2016; Sun *et al.* 2020).

Maximizing the potential of plants in terms of productivity and quality through bioorganic methods, such as the application of bioorganic fertilizers, biostimulants, or bioregulators, offers a potential solution to this crisis (Rouphael *et al.* 2018; Gakhokidze 2019; Avkopashvili *et al.* 2022). Although existing options are not without flaws (Yakhin *et al.* 2017), there is a rapidly growing market to produce these products (Rademacher 2015).

This study focused on a new non-toxic bioregulator, bioragi, developed by Prof. Ramaz Gakhokidze using principles of the emerging field of agrobioorganic chemistry (Gakhokidze 2016; Avkopashvili *et al.* 2022). With its unique composition - a glyceride containing 41.4% carbon, 6.5% hydrogen, and 52.1% oxygen—bioragi has no analogues. Initial applications have demonstrated its ability to yield ecologically pure, high-quality harvests while minimizing chemical inputs (Avkopashvili *et al.* 2022). Bioragi belongs to a class of biostimulators known as bioenergy activators (Mire *et al.* 2016), which influence plant productivity and internal regulatory systems in various ways (Yakhin *et al.* 2017; Baltazar *et al.* 2021). They impact metabolic processes, such as respiration, photosynthesis, and the synthesis of key biomolecules such as proteins and carbohydrates (Zulfiqar *et al.* 2019). They also increase the activity of several enzymes, for example katalases and peroxidases, and change the penetrability of cellular membranes for water, mineral, and organic substances (Vasconcelos *et al.* 2019; Priadkina *et al.* 2020). Furthermore, bioenergy activators exhibit strong antioxidant properties, thus mitigating the adverse effects of agricultural chemicals on human health (Vlaicu *et al.* 2024; Drobek *et al.* 2019). Through activation of plant-internal regulatory mechanisms, they enable homeostasis and thus adaptation to extreme conditions (Rouphael *et al.* 2018). The use of bioenergy activators has led to significant increases in crop yields (Amoanimaa-Dede *et al.* 2022), and enhance the nutritional value of the produce, with improvements in protein composition, and the contents of amino acids, vitamins, and micronutrients (Nicholson *et al.* 2015; Di Vittori *et al.* 2018).

A particularly intriguing example of bioragi application is the more than four-fold increase in sugar beet productivity, *i.e.*, from a global average of 7 tons of crystal sugar per hectare (50 tons of harvest with a sugar content of 14%) (Kaffka *et al.* 2014; Ingole *et al.* 2018) to bioragi treated sugar beet harvest 32 tons per hectare (143 tons of harvest with a sugar content of 22.5%) (Gakhokidze 2006; Avkopashvili *et al.* 2022). Sugar beet (*Beta vulgaris*) is a biennial, commercially important root crop that secures nearly 20% of global sugar production (Hoffmann *et al.* 2018). It is grown mainly under temperate environment (Artrua *et al.* 2018), but also under dry and semi-dry climatic conditions (Gholamreza *et al.* 2014; Khan *et al.* 2018). The basis for this crop's commercial use in sugar production is its ability to store large amounts of sucrose in its roots (McGrath *et al.* 2015). Considering the potential cultivation of sugar beet in or near the mining areas, the objective of the present study was to evaluate whether bioragi treatment would decrease the accumulation of potentially toxic trace elements in the sugar beet root, thereby producing a healthier product in addition to increasing biomass production.

MATERIALS AND METHODS

Plant Growth and Sampling

Plant growth studies were conducted under environmental conditions in a field near the gold and copper mining area in Balichi, Georgia. The soils surrounding this enterprise are contaminated mainly with the following metals Ti, V, Cr, Co, Ni, Cu, Zn, As, Rb, Sr, Mo, Cd, Cs, Ba, Pt, Au, Pb, and Th (Avkopashvili *et al.* 2017). Therefore, the aim of this research was to investigate the uptake of these elements by sugar beets. The soil at the site is a chernozem with a pH between 6.4 to 7.5. A 100 m² plot was prepared for the experiment (Fig. 1). Sugar beet seeds were soaked in a 0.15 g L⁻¹ solution of bioragi for 24 h. Bioragi was not used for the control plant samples. Seeds were sown in April and harvested in June, July, and August. Plants were not irrigated during growth, *i.e.*, they received atmospheric precipitation only.



Fig. 1. A) Field experiment; B) Harvested Sugar beet, the small one is the control Sugar beet, and the big one is the Sugar Beet with bioragi

The study was conducted dynamically, with different parts of the plant analyzed separately. Two groups were compared: a control group and a group treated with bioragi. Bioragi was used as a bioenergetic activator to enhance the plant's ability to accumulate substances.

Soil Sampling

Soil samples were taken at zero to 5 cm, and 30 to 35 cm in depth, using scoop samplers that were washed between each sampling. The study area was divided into regular grids of 10 × 10 m, where a sample was collected at five points, they were mixed, and a composite sample was prepared. The composite soil sample was placed into a polyethylene bag, labeled, and transported into the laboratory. The soil samples were oven-dried at 50 °C for 24 h, followed by grinding and sieving using a 0.18 mm sieve.

Plant Samples, Soil Digestion, and Elemental Analysis

Plant samples, dried at 40 °C, were digested using 5 mL nitric acid per gram of dry sample. After 3 h in the hot water bath, the solution was filtered through a 45 µm Whatman filter paper. Trace metals in the digest were determined using ICP-MS. To determine the concentrations of metals in soil samples, 5 mL of 65% HNO₃ (trace metal grade) were added to 1.0 g of soil in a 50 mL volumetric flask. The flask was heated in a water bath (100 °C) for 2 h followed by 15 min cooling and then filtering through a Whatman 0.45 µm filter paper into another 50 mL volumetric flask. The filtrate was made up to volume and analyzed for trace metals by ICP-MS. About 10% of the samples were analyzed in duplicate.

Calculations of Accumulation Coefficient

In order to evaluate element uptake in bioragi-treated and control sugar beets, accumulation coefficients (AC) were used as shown in Eq. 1.

$$AC = C_{\text{plant}} / C_{\text{soil}} \quad (1)$$

Here, C_{plant} is the concentration of the element in the plant, and C_{soil} is concentration of the element in the soil. At $AC > 1$, the plant has a good ability to accumulate the element, and at $AC < 1$ the plant did not show a good ability to accumulate the element.

The translocation factor (TF) is calculated using the following Eq. 2,

$$TF = C_{\text{shoot}} / C_{\text{root}} \quad (2)$$

where C_{shoot} and C_{root} are the concentrations of the element in the above-ground parts (leaf, flower, stem) and under-ground parts (root, bulb) of the plant. When $TF > 1$, that means that the plant has the ability to efficiently translocate (move) any element from the roots to its above-ground parts.

RESULTS

Indicators in the Plant and Soil During the Study Period

The effects of biostimulant bioragi on two plant organs, namely the leaf and root, were investigated in the experiment. The root yield (RY) was determined directly at the plot and calculated per hectare. The bioragi-treated sugar beet parameters are presented in Table 1. The physicochemical characteristics of the studied soil are presented in Table 2.

Table 1. Influence of Bioragi on Increase of Total Size of Leaf and Sucrose in Sugar Beet

	Average No. of Leaves on One Plant	Average Size of One Leaf (cm ²)	Total Size of One Leaf (cm ²)	Sucrose (%)	Plant Biomass in June (g)	Plant Biomass in July (g)	Plant Biomass in August (g)
Control	16	205	3280	10.3	70	109	173
Bioragi	58	350	20300	22.5	90	134	292

Table 2. Soil Content - Total Nitrogen and Carbon

Sample ID	Total %N	Total %C	C/N Ratio
Soil Surface	0.277	2.839	10.25
Soil Bottom	0.242	2.490	10.29

The experiment confirmed the stimulating effect of the bioragi treatment on biomass production and bulb sugar content of sugar beet (Tables 1 to 2). In particular, on average the bioragi-treated plants had 42 more leaves than the control plant, and the average leave size of bioragi-treated plants exceeded that of control plants by 145 cm² (Table 1). Furthermore, the concentration of sucrose in the bulb of bioragi-treated sugar beets was more than twice than that in the bulb of the control plants. Overall, these data translate into about 4 times higher sugar production through bioragi treatment.

The main objective of this study was to assess whether treatment of sugar beet seeds with the biostimulator bioragi would increase biomass production and sugar content. In addition, this study evaluated the decreases in the plant uptake of potentially toxic trace metals from the soil.

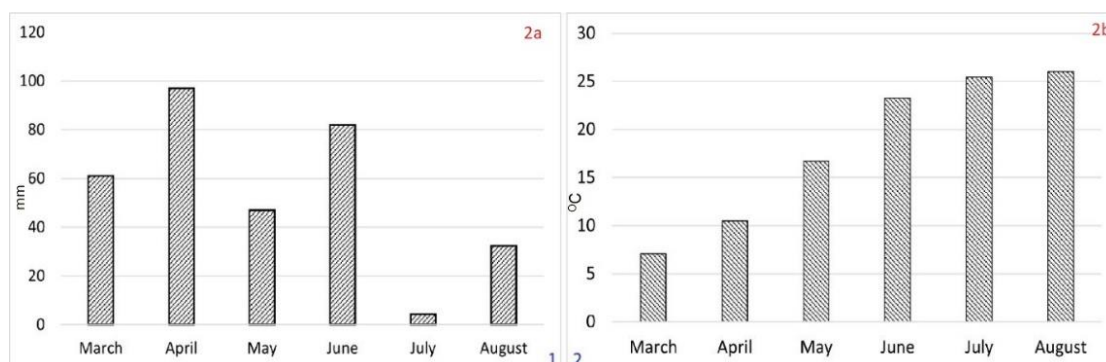


Fig. 2. Climatic data from the beginning day of the experiment to the final day of the experiment: a) Monthly sum of precipitation (mm) by months; b) Average temperature (°C) by months.

Daily records of precipitation and temperature during the experimental period from March to August revealed the highest amounts of precipitation in April and June, and the lowest in July.

When observing the plants during the study, it was found that compared to the control plant, the life of the bioragi-treated plant was extended by about 15 to 20 days. Therefore, it should be noted that bioragi not only makes the plant more resistant to stress factors (cold or heat), but also prolongs its viability. In an earlier study, it was shown that bioragi treatment led to a 33% lower cadmium uptake, a 20% lower copper uptake, and a 23% lower zinc uptake by sugar beet compared to control plants (Avkopashvili *et al.* 2022). The results from the present study reveal that, considering the data from the last sampling in August, bulbs of bioragi-treated plants had lower concentrations of most elements (Table 3). Notable exceptions are Cu, Mo, and Cd with equal concentrations in treated and control plants, and Sr with higher concentrations in the treated plants. Considering the time course, generally lower concentrations of several elements were found in plants sampled in July. This may be related to a reduced element uptake caused by stress imposed on the plants by the transition from the moist conditions in June stimulating plant growth, to the dry conditions in July (Fig. 2).

Table 3. Contents of Chemical Elements in Sugar Beet

	June					July					August				
	Soil A* (µg/kg)	Soil S* (µg/kg)	Soil B* µg/kg	Sugar Beet C* (µg/kg)	Sugar beet BT* (µg/kg)	Soil A* (µg/kg)	Soil S* (µg/kg)	Soil B* (µg/kg)	Sugar Beet C* (µg/kg)	Sugar beet BT* (µg/kg)	Soil A* (µg/kg)	Soil S* (µg/kg)	Soil B* (µg/kg)	Sugar Beet C* (µg/kg)	Sugar beet BT* (µg/kg)
Ti	197.52	178.81	216.23	56.67	67.75	215.69	258.02	239.39	46.34	53.34	264.29	218.41	227.81	126.68	65.47
V	36.68	35.11	38.26	1.47	2.70	38.49	3452	35.95	1.60	1.21	43.12	34.81	37.113	12.96	4.59
Cr	26.07	25.29	26.84	5.23	5.23	29.15	24.934	27.22	4.06	4.72	38.49	25.11	27.03	15.89	7.99
Co	7.34	7.34	7.34	0.53	0.78	9.78	7.43	7.65	0.49	0.60	11.38	7.39	7.49	2.31	1.09
Ni	25.88	25.90	25.86	8.13	6.29	24.34	26.38	27.27	5.88	6.64	26.75	26.14	26.56	16.53	9.77
Cu	14.28	14.65	13.89	25.60	26.59	16.82	13.42	13.59	24.00	22.89	20.47	14.03	13.75	36.25	39.44
Zn	32.07	32.14	31.93	110.30	80.51	39.43	33.84	33.41	87.64	112.50	49.53	32.99	32.70	81.02	59.62
As	6.92	7.05	6.79	0.48	0.56	11.65	5.66	6.13	0.45	0.27	9.83	6.39	6.46	2.46	0.85
Rb	24.32	22.92	25.72	28.38	36.58	29.57	29.05	32.728	32.31	52.27	29.41	25.99	29.22	50.38	43.01
Sr	155.74	149.63	161.85	89.74	86.88	179.25	69.27	72.79	91.42	116.22	197.62	109.45	117.31	85.35	208.36
Mo	0.41	0.42	0.47	9.14	6.43	1.36	0.32	0.21	11.35	8.75	0.94	0.37	0.31	4.43	3.81
Cd	0.19	0.19	0.19	0.62	0.50	2.42	0.19	0.218	0.44	0.39	2.59	0.197	0.21	0.39	0.38
Cs	1.37	1.30	1.44	0.06	0.09	1.16	1.51	1.64	0.07	0.07	1.74	1.41	1.54	0.44	0.19
Ba	366.00	391.21	340.81	486.82	150.91	403.51	346.73	364.25	384.21	110.95	346.57	368.98	352.53	222.05	136.22
Pt	0.08	0.06	0.09	0.08	0.16	0.13	0.06	0.09	0.06	0.03	0.12	0.065	0.09	0.02	0.04
Au	0.04	0.04	0.04	0.23	0.11	0.09	0.02	0.03	1.18	0.11	0.06	0.032	0.04	0.07	0.06
Pb	6.56	6.69	6.46	0.73	0.97	7.45	8.118	6.54	0.83	0.79	7.04	7.4	6.51	2.82	1.71
Th	3.06	3.03	3.09	0.14	0.18	4.31	3.33	3.49	0.09	0.10	5.42	3.18	3.29	0.38	0.22

A* - Average; S* - Surface; B* - Bottom; C* - Control; and BT* - Bioragi-treated.

Table 4. Amount of AC- (Accumulation coefficient) and TF- (translocation factor) in Control and Bioragi-treated Sugar Beet in June, July, and August

	June				July				August			
	Control		Bioragi		Control		Bioragi		Control		Bioragi	
	AC	TF	AC	TF	AC	TF	AC	TF	AC	TF	AC	TF
Ti	0.29	0.86	0.34	0.94	0.23	0.64	0.27	0.76	0.64	3.21	0.33	1.60
V	0.04	0.50	0.07	0.69	0.04	0.35	0.03	0.42	0.35	5.31	0.13	1.40
Cr	0.20	0.62	0.20	0.93	0.16	0.66	0.18	0.74	0.61	3.29	0.31	1.58
Co	0.07	0.72	0.11	0.88	0.07	0.70	0.08	0.92	0.32	4.55	0.15	1.66
Ni	0.31	1.40	0.24	0.80	0.23	0.49	0.26	0.66	0.64	3.94	0.38	1.51
Cu	1.79	0.99	1.86	1.02	1.68	0.70	1.60	0.86	2.54	1.97	2.76	1.35
Zn	3.44	1.09	2.51	0.85	2.73	0.76	3.51	1.33	2.53	1.12	1.86	0.93
As	0.07	0.65	0.08	0.74	0.07	0.84	0.04	0.31	0.36	4.73	0.12	1.32
Rb	1.17	2.30	1.50	3.15	1.33	2.98	2.15	2.99	2.07	4.12	1.77	2.72
Sr	0.58	1.72	0.56	1.56	0.59	1.64	0.75	1.82	0.55	1.91	1.34	1.77
Mo	22.02	7.75	15.49	9.76	27.36	12.93	21.10	8.31	10.67	8.35	9.18	6.86
Cd	0.41	1.91	0.33	1.94	0.29	2.12	0.26	2.06	0.26	1.96	0.25	2.15
Cs	0.04	0.91	0.07	0.99	0.05	0.81	0.05	1.21	0.32	5.33	0.14	2.06
Ba	1.33	1.10	0.41	1.41	1.05	1.09	0.30	1.98	0.61	2.01	0.37	1.43
Pt	1.02	0.20	1.89	3.71	0.70	0.79	0.41	1.93	0.27	0.92	0.47	0.03
Au	6.10	3.34	2.88	3.64	30.83	8.58	2.97	0.52	1.90	1.41	1.66	1.09
Pb	0.05	1.30	0.06	1.43	0.06	1.19	0.05	1.52	0.19	4.91	0.11	2.53
Th	0.04	0.54	0.06	1.07	0.03	0.48	0.03	0.57	0.12	4.40	0.07	2.07

Accumulation coefficient (AC) and translocation factor (TF) were calculated in sugar beet (Table 4). Based on samples taken in June, sugar beet showed good accumulation and translocation capacities for Cu, Zn, Rb, and Mo. The values of AC and TF for Cu were found to be >1 in bioragi-treated sugar beet. The best accumulation capacity (3.44) and translocation factor (1.09) of Zn were found in the control sugar beet. The AC value for Mo accumulation in the control sugar beet is 22, which is a very good result, although it should also be noted that the Mo content in the soils was low. Mo is an essential nutrient; thus the high AC may effectively reflect a high demand of the plants.

Plant samples taken in July showed good accumulation and translocation capacities again for Cu, Zn, Rb, and Mo, and in some cases for Ba element. However, in the control sugar beet sample, for Cu and Zn indicated $TF < 1$. For Zn, bioragi-treated sugar beet showed good results of $TF = 1.33$.

Similarly, in the samples taken in the month of August, sugar beet plants showed good AC and TF capacities for Cu, Zn, Rb, and Mo. It is interesting that the TF of the control and bioragi-treated plants exceeds 1.0 for almost all elements, which was not observed in the samples taken in previous months (Table 5), although in this case the TF of almost all elements in the control sugar beet exceeds the TF of the bioragi-treated sugar beet.

Figures 3 c and d shows that organs of bioragi-treated sugar beet absorbed 2.5 times less Ba element compared to the control plants. In terms of Zn accumulation, the Zn content in the leaf, stem, and root of the control sugar beet was the highest in June, and its concentration decreased in July and August, while the highest concentration was observed in the peel in July and the lowest in June and August. As for the bioragi-treated sugar beet, more Zn was accumulated by the leaf, stem, and root in July compared to June and August. As for the peel (Fig 3 g and h), the Zn content was the same in all three periods. The accumulation of As from the soil was quite low. In the leaf, stem, and peel of the control sugar beet, the accumulation of As was relatively higher in the August, while a decrease was observed in $June < July < August$ in the root, both in the control and in the bioragi-treated plants. But at the same time, if the amounts of elements in the plant in the months of June-July-August were compared, it can be seen that the amount of elements in the sugar beet in August was higher than that in the samples taken in other months. This fact is explained by the event that with the increase of time, the plant absorbs elements from the soil in larger quantities, and with the increase in temperature in the dry summer period, when the soil becomes exhausted and dries up, it is difficult for the roots of the plant to absorb nutrients from the exhausted and dry soil, as well as various chemical elements with them. It turns out that the root of the plant absorbs nutrients from the soil in a smaller amount, although elements are moved to the stem and leaves with the same intensity.

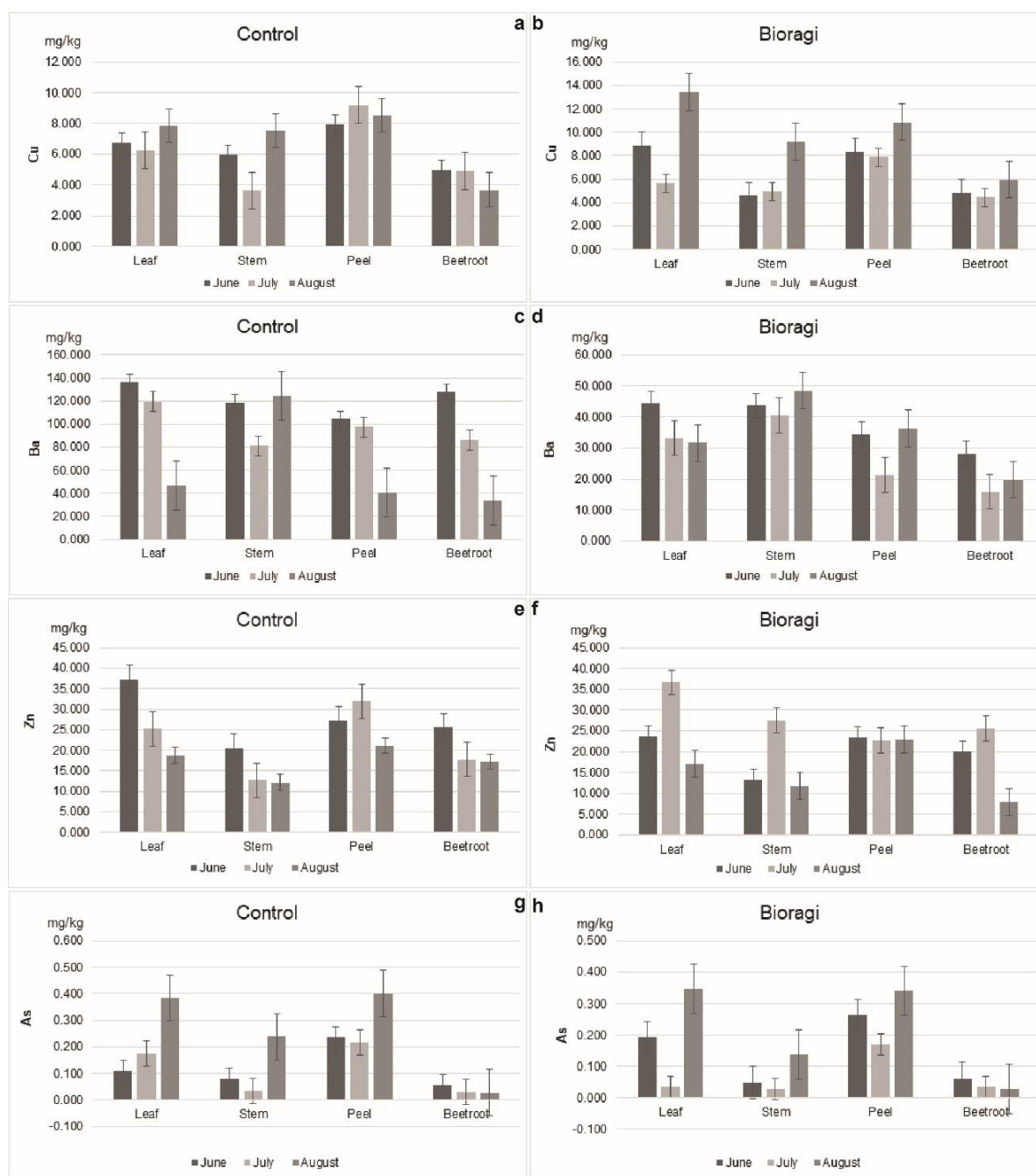


Fig. 3. Control and bioragi-treated sugar beet contents of some metals in the leaf-stem-peel-beetroot in June, July, and August. (a) Control sugar beet copper (Cu) concentration, (b) Biostimulant Bioragi treated sugar beet copper concentration, (c) Control sugar beet barium (Ba) concentration, (d) Biostimulant Bioragi treated sugar beet barium (Ba) concentration, (e) Control sugar beet zinc (Zn) concentration, (f) Biostimulant Bioragi treated sugar beet Zinc (Zn) concentration, (g) Control sugar beet arsenic (As) concentration, (h) Biostimulant Bioragi treated sugar beet arsenic (As) concentration.

DISCUSSION

The biostimulant bioragi was used in this study to assess its effect on sugar beet growth and heavy metal accumulation from the soil. Compared to the control plants, those treated with the bioactivator showed no significant difference in the concentration of some elements. One possible explanation is that bioragi limits the accumulation of certain trace elements, such as Cu, Zn, and Cd. The study results indicate that bioragi can be used to reduce the uptake of both toxic and less toxic metals by plants. Therefore, the application of bioragi is also suitable for cultivating food crops on contaminated soils, as it decreases the bioavailability and plant absorption of trace metals.

The reduction in Cu, Zn, and Cd uptake in bioragi-treated plants aligns with previous studies that suggest certain biostimulants can alter root membrane permeability, thereby limiting metal absorption. For instance, research by Bulgari *et al.* (2019) and Calvo *et al.* (2014) indicates that biostimulants such as protein hydrolysates and microbial inoculants can modify root architecture and cell wall composition, reducing heavy metal translocation. Similarly, a study by Rouphael and Colla (2020) found that specific plant growth-promoting rhizobacteria (PGPR) can enhance plant resilience while limiting excessive metal uptake by modulating nutrient transporters.

However, unlike the findings of Vives-Peris *et al.* (2020), which reported an increase in Zn accumulation due to enhanced root exudation in plants treated with certain organic biostimulants, the present study found a reduction in Zn uptake. This suggests that the mechanism of bioragi's influence may differ from other bioactivators.

From June to July, the concentration of elements in the bulb/peel of the plant was relatively higher than in the above-ground parts (stems and leaves). However, by August, the element content in the root/peel had decreased and was lower than that in the stems and leaves. In sugar beet treated with bioragi, copper accumulated more in the leaves than in any part of the control plant, including the leaves, stems, peel, and roots. Notably, the overall copper concentration decreased compared to June and July, which may be partially attributed to increased climatic precipitation (Fig. 2). In contrast to copper, barium (Ba) was absorbed differently across plant organs. In June, Ba was absorbed by the leaf, stem, peel, and root of both bioragi-treated and control sugar beet in larger quantities, while in July and August, the concentration of Ba decreased. From June to August the concentration of Ti, V, Cr, Co, Ni and Mo decreased. Meanwhile, the following elements such as Cs, Pt, Au, Pb, Th did not show significant absorption variation. A notable observation was the fluctuation in metal concentrations in both soil and sugar beet tissues over time. For instance, Cu concentrations in soil remained relatively stable across the months, ranging from 13.4 to 20.5 µg/kg. However, in sugar beet tissues, Cu accumulation increased from 25.60 µg/kg in June to 39.44 µg/kg in August. This trend suggests a progressive accumulation of Cu in plant tissues over time, possibly due to increased root activity or prolonged exposure. Similar patterns were observed for Zn, where its concentration in sugar beet varied from 80.51 µg/kg in June to 59.62 µg/kg in August, indicating dynamic uptake mechanisms influenced by plant growth and environmental factors. Interestingly, the uptake of Cd remained relatively low but followed a decreasing trend in plant tissues, from 0.62 µg/kg in June to 0.39 µg/kg in August. This aligns with studies suggesting that biostimulants can reduce Cd bioavailability by altering root membrane permeability (Bulgari *et al.* 2019; Ergül *et al.* 2024). Moreover, elements such as Sr and Rb showed significant variations, with Sr increasing from 86.88 µg/kg in June to 208.36 µg/kg in

August, while Rb followed a similar increasing trend in June, peaking at 50.38 $\mu\text{g/kg}$ in July before slightly decreasing in August.

Research results have shown that a plant such as sugar beet can be used for sources of some trace metals. As a result of this research, it was found that sugar beet accumulates such trace metals as Cd, Cu, and Zn in the largest amount in the month of August, compared to other (June and July) periods. The reason for this is that the longer a plant leaf, the more elements it can accumulate. When observing the study of different individual parts of plants, it appeared that in the case of such a bulbous plant as beetroot, the accumulation of elements occurs mostly in the peel of the plant compared to other parts. Studies have shown that the growth of a plant mass does not affect the amount of element absorption by it. In addition, our results indicate that the use of the bioactivator bioragi to enhance the plant's accumulation capacity may not always be appropriate, as in some cases it limits the uptake of certain elements by the plant.

The advantage of the bioactivator was shown in the fact that the biomass of sugar beet, its life span, and the ability to retain elements in it for a long time increased. Specifically, if the control plant began to wither, in the case of the plant with bioactivator, the life of the plant and the ability to retain elements in it was extended by one month. The amount of sucrose accumulated in the sugar beet root using the bioactivator bioragi was 22.2%, and the sucrose content of the control sugar beet was 10.3%. This is a very important result in the case of industrial production of sucrose, which will be directly proportional to the increase in sugar production.

While bioragi reduced the uptake of Cu, Zn, Ba, Ti, V, Cr, Co, Ni, Mo and Cd, its impact on other trace elements such as Sr and As varied. A detailed breakdown is provided in Table 3, summarizing the percentage change in element accumulation compared to control plants. Notably, arsenic (As) concentrations decreased by 12%. The application of bioragi led to a reduction in the uptake of certain toxic elements such as Cd, As, and Pb. Compared to the control, Cd uptake was reduced by approximately 18% to 50% across the three months, supporting the hypothesis that bioragi limits toxic metal translocation to edible plant tissues. This finding aligns with previous studies that have shown biostimulants can mitigate heavy metal stress in crops (Rouphael and Colla 2020). However, the absorption of elements such as Sr, which can pose potential health risks even at low concentrations, increased significantly, necessitating further investigation into bioragi's influence on alkaline earth metal dynamics.

Environmental conditions, particularly drought and temperature variations, significantly influenced plant growth and metal uptake. The extended lifespan and enhanced biomass of bioragi-treated plants suggest increased drought resistance. This aligns with studies by Gakhokidze (2019), who found that biostimulants improve plant resilience to abiotic stress. The increased biomass, however, did not directly correlate with higher element absorption, indicating that Bioragi's primary effect was on plant health rather than enhanced metal uptake.

This study should help decision makers consider this area in agricultural development of plans and pollution prevention and remediation. However, this study had a fairly limited number of samples, which introduces a level of uncertainty to the results. More studies regarding biological monitoring and pollution in food chains should be performed in the future.

CONCLUSIONS

1. This study showed that bioragi significantly reduced the accumulation of certain trace metals, such as Cu, Zn, and Cd, in sugar beets by an average of 18% to 50%. This indicates that bioragi can be effectively used to limit the uptake of both toxic and less toxic metals in plants, making it suitable for growing food products on contaminated soils.
2. Additionally, bioragi enhances the biomass and lifespan of sugar beet plants, improving their resistance to stress factors such as climatic conditions. Notably, the sucrose content in sugar beet roots treated with bioragi was significantly higher, at 22.2%, compared to 10.3% in control plants. This increase in sucrose content is highly advantageous for industrial sugar production, directly correlating with increased sugar yield.

Overall, the bioactivator bioragi demonstrates potential for improving crop quality and yield while mitigating the negative impacts of trace metal accumulation in plants.

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REFERENCES CITED

- Amoanimaa-Dede, H., Su, C., Yeboah, A., Zhou, H., Zheng, D., and Zhu, H. (2022). "Growth regulators promote soybean productivity: A review," *Plant Biology. Peer J.* 10, article no. e12556. DOI: 10.7717/peerj.12556.
- Artrua, S., Lassoisa, L., Vancutsemb, F., Reubensc, B., and Garré, S. (2018). "Sugar beet development under dynamic shade environments in temperate conditions," *European Journal of Agronomy* 97, 38-47.
- Avkopashvili, M., Avkopashvili, G., Avkopashvili, I., Asanidze, L., Matchavariani, L., Gongadze, A., and Gakhokidze, R. (2022). "Mining-related metal pollution and ecological risk factors in South Eastern Georgia," *Sustainability* 14, article 5621. DOI: 10.3390/su14095621
- Avkopashvili, G., Avkopashvili, M., Gongadze, A., Gakhokidze, R., Avkopashvili, I., and Asanidze, L. (2022). "Influence of biostimulants on the cadmium, zinc and copper accumulation potential of the sugar beet (*Beta vulgaris*) and analysis ANOVA and accumulation coefficient," *Carpathian Journal of Earth and Environmental Sciences* 17(1), 149-157. DOI: 10.26471/cjees/2022/017
- Avkopashvili, G., Avkopashvili, M., Gongadze, A., and Gakhokidze, R. (2017). "Eco-monitoring of Georgia's contaminated soil and water with heavy metals," *Carpathian Journal of Earth and Environmental Sciences* 12, 595-604.
- Baltazar, M., Correia, S., Guinan, K. J., Sujeeth, N., Bragança, R., and Gonçalves, B. (2021). "Recent advances in the molecular effects of biostimulants in plants: An overview," *Biomolecules* 11, article 1096. DOI:10.3390/biom11081096

- Bulgari, R., Franzoni, G., and Ferrante, A. (2019). "Biostimulants application in horticultural crops under abiotic stress conditions," *Agronomy* 9(6), 306. DOI: 10.3390/agronomy9060306
- Cai, S., Zhu, H., Wang, J., Yu, T., Qian, X., and Shan, Y. (2016). "Fertilization impacts on green leafy vegetables supplied with slow-release nitrogen fertilizers," *J. Plant Nutr.* 39(10), 1421-1430. DOI:10.1080/01904167.2015.1050508.
- Calvo, P., Nelson, L., and Kloepper, J. W. (2014). "Agricultural uses of plant biostimulants," *Plant and Soil* 383, 3-41. DOI: 10.1007/s11104-014-2131-8
- Di Vittori, L., Mazzoni, L., Battino, M., and Mezzetti, B. (2018). "Pre-harvest factors influencing the quality of berries," *Sci. Hortic.* 233, 310-322.
- Di Sario, C., Rossi, G., and Bernardini, P. (2025). "Plant biostimulants to enhance abiotic stress resilience in crops," *International Journal of Molecular Sciences* 26(3), 1129. DOI: 10.3390/ijms26031129
- Drobek, M., Fra, M., and Cybulska, J. (2019). "Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress: A review," *Agronomy* 9, 335.
- Ergül, H. A., and Kravkaz Kuşçu, İnci S. (2024). "Variations in Sr, Tl, and V concentrations at copper mining sites based on soil depth, plant species, and plant organ," *BioResources* 19(4), 7931-7945.
- FAO, Food and Agriculture Organization of the United Nations (2022). Available online: <https://www.fao.org/newsroom/detail/new-un-report-warns-that-global-food-crises-likely-to-increase-in-the-future-without-wider-systemic-change/en> (accessed on 3 March 2022).
- Gakhokidze, R. (2006). "Bioragi" vitamin for plant," *Liberator*. 10, (In Georgian).
- Gakhokidze, R. (2016). "The method of bio-activation of seedlings and plants," Patent. Issuing organization: Ivane Javakhishvili Tbilisi State University. Registration number: P6 463, Year of Issue (2016). (In Georgian).
- Gakhokidze, R. (2019). "Bio-organic green revolution," *The Monograph* pp 1-138.
- Gholamreza, H., Farnia, A., Rahnamaeian, M., and Shaban, M. (2014). "Effect of different biofertilizers and irrigation closed time on some agronomic characteristics of sugar beet (*Beta vulgaris* L.)," *Int. J. Adv. Biol. Biom. Res.* 2(8), 2375-2380.
- Hoffmann, C. M. and Kenter, C. (2018). "Yield potential of sugar beet – Have we hit the ceiling?," *Front. Plant Sci.* 9, 289. DOI:10.3389/fpls.2018.00289.
- Ingole, V., Wagh, A., Nagre, P., and Bharad, S. (2018). "Effect of combination of organic manure and biofertilizer for better growth and yield of beetroot (*Beta vulgaris* L.)," *International Journal of Chemical Studies* 6 (5), 1222-1225.
- Kaffka, S. R., and Grantz, D. A. (2014). *Sugar Crops, Encyclopedia of Agriculture and Food Systems*, Reference Module in Food Science, pp 240-260.
- Khan, I., Iqbal, M., and Hashim, M. M. (2018). "Physicochemical characteristics and yield of sugar beet (*Beta vulgaris* L.) cv. 'California-Kws' influenced with irrigation intervals," *Sarhad J. Agric.* 35, 57-69.
- Knapp, S., and Heijden, M. G. A. (2018). "A global meta-analysis of yield stability in organic and conservation agriculture," *Nat. Commun.* 9, 3632. DOI:10.1038/s41467-018-05956-1.
- Kopittke, P. M., Menzies, N. W., Wang, P., McKenna, B. A., and Lombi, E. (2019). "Soil and the intensification of agriculture for global food security," *Environment International* 132, Article no. 105078.

- Lichtfouse, E., Navarrete, M., Debaeke, P., Souchère, V., Alberola, C., and Ménassieu, J. (2009). "Agronomy for sustainable development – A review," *Agronomy for Sustainable Development* 29, 1-6. DOI: 10.1051/agro:2008054.
- McGrath, J. M., and Townsend, B. J. (2015). "Sugar beet, energy beet, and industrial beet," in: *Industrial Crops: Handbook of Plant Breeding*, V. M. V. Cruz and D. A. Dierig (eds.), Springer, New York, USA.
- Mire, G. L., Nguyen, M. L., Fassotte, B., Du Jardin, P., Verhegeen, F., and Delaplace, P. (2016). "Implementing plant biostimulants and biocontrol strategies in the agroecological management of cultivated ecosystems, A review," *Biotechnology, Agronomy, Society and Environment* 20(S1), 299-313.
- Naher, U. A., Biswas, J. C., Maniruzzaman, M., Khan, F. H., Sarker, M. I. U., Jahan, A., Hera, M. H. R., Hossain, M. B., Islam A., Islam M. R., *et al.* (2021). "Bio-organic fertilizer: A green technology to reduce synthetic n and p fertilizer for rice production," *Front. Plant Sci.* 12, article no. 602052. DOI: 10.3389/fpls.2021.602052
- Nicholson, N., Zvomuya, J. F., Lisette-Ross, N. C., and Badiou, P. (2015). "Biomass, nutrient, and trace element accumulation and partitioning in cattail (*Typha latifolia* L.) during wetland phytoremediation of municipal biosolids," *J. Environ. Qual.* 44, 1541-1549. DOI: 10.2134/jeq2015.02.0064.
- Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., and Hens, L. (2016). "Chemical pesticides and human health: The urgent need for a new concept in agriculture," *Front. Public Health.* 4, article 148. DOI:10.3389/fpubh.2016.00148.
- Priadkina, G. O. (2020). "Influence of trace elements, applied in classical and nano forms, on photosynthesis of higher plants in relation to enhancement of crop productivity," *Agricultural Science and Practice.* 7(3), P. 71-86.
- Rademacher, W. (2015). "Plant growth regulators: Backgrounds and uses in plant production," *J. Plant Growth Regul.* 34, p. 845-872. DOI: 10.1007/s00344-015-9541-6
- Rouphael, Y., Spichal, L., Panzarova, K., Casa, R., and Colla, G. (2018). "High-throughput plant phenotyping for developing novel biostimulants: From lab to field or from field to lab?," *Front. Plant Sci.* 9, p. 1-19.
- Rouphael, Y., and Colla, G. (2018). "Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture," *Front. Plant Sci.* 9, 1-24. DOI:10.3389/fpls.2018.01655.
- Rouphael, Y., and Colla, G. (2020). "Biostimulants in agriculture," *Frontiers in Plant Science* 11, 40. DOI: 10.3389/fpls.2020.00040
- Şevik, H., Yildiz, Y., and Özel, H. B. (2024). "Phytoremediation and long-term metal uptake monitoring of silver, selenium, antimony, and thallium by black pine (*Pinus nigra* Arnold)," *BioResources* 19(3), 4824-4837.
- Sun, B., Gu, L., Bao, L., Zhang, S., Wei, Y., and Bai, Z. (2020). "Application of biofertilizer containing *Bacillus subtilis* reduced the nitrogen loss in agricultural soil," *Soil Biol. Biochem.* 148, 107911-107921. DOI:10.1016/j.soilbio.2020.107911
- Tsiafouli, M. A., Thébault, E., Sgardelis, S. P., Ruiter, P. C., Putten, W. H., Birkhofer, K., Hemerik, L., Vries, F. T., Bardgett, R. D., Rady, M. V., *et al.*, (2015). "Intensive agriculture reduces soil biodiversity across Europe," *Glob. Chang. Biol.* 21, 973-985. DOI: 10.1111/gcb.12752
- Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., and Phung, D.T. (2021). "Agriculture development, pesticide application and its impact on the environment," *Int. J. Environ. Res. Public Health* 18, 1112. DOI: 10.3390/ijerph18031112

- Vlaicu, P. A., and Untea, A. E. (2024). "Application of natural antioxidants from fruits waste for improving egg quality characteristics," *Appl. Sci.* 14, article 10437. DOI: 10.3390/app142210437
- Vasconcelos, A. C. F., and Chaves, L. H. G. (2019). "Biostimulants and their role in improving plant growth under abiotic stresses," in: *Biostimulants in Plant Science*, IntechOpen, DOI: 10.5772/intechopen.88829
- Vives-Peris V., de Ollas C., Gómez-Cadenas A., and Pérez-Clemente R. M. (2020). "Root exudates: From plant to rhizosphere and beyond," *Plant Cell Reports* 39, 3-17.
- Yakhin O.I., Lubyantov, A.A., Yakhin, I.A., and Brown, P.H. (2017). "Biostimulants in plant science: A global perspective," *Front. Plant Sci.* 7, article 2049. DOI:10.3389/fpls.2016.02049
- Yang, Y., Zhou, S., Y. Xing, G. Yang, M. You. (2023). "Impact of pesticides exposure during neurodevelopmental period on autism spectrum disorders – A focus on gut microbiota," *Ecotoxicol. Environ. Saf.* 260 article 115079. DOI: 10.1016/j.ecoenv.2023.115079
- Yiğit, N. (2024). "Determination of sixteen woody species' ability to sequester Sr, Mo, and Sn pollutants," *BioResources* 19(4), 7842-7855. DOI: 10.15376/biores.19.4.7842-7855
- Zulfiqar, F., Younis, A., and Abideen, Z. (2019). "Bioregulators can improve biomass production, photosynthetic efficiency, and ornamental quality of *Gazania rigens* L.," *Agronomy* 9, article 773.

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