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Mass Spectrometric Analysis of Elements in Zoohumus Based on *Tenebrio molitor*

Sayyora S. Salomova[1], Nortoji A. Khujamshukurov[2, 5*], Xurshid N. Isaboyev[2], Dilorom B.Turabekova[3], Dilafruz Kh. Kuchkarova[4], Alvina Farooqui[5] and Tripath Gyanendra[5]

[1]*Karshi State University, Uzbekistan,*
[2]*Tashkent Institute of Chemical Technology, Tashkent, Uzbekistan*
[3]*Gilistan State University, Gulistan, Uzbekistan*
[4]*Tashkent University of Architecture and Civil engineering, Tashkent, Uzbekistan*
[5]*Integral University, Lucknow, India*

**Corresponding author*

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In this scientific article, the elemental composition of various fractions of zoohumus obtained from the feeder insect *Tenebrio molitor* was studied using the mass spectral analysis method. The study compared purified (fine fraction) zoohumus obtained from larvae, untreated (coarse fraction) zoohumus obtained from larvae, purified (1.0 mm sieve) zoohumus obtained from beetles, zoohumus obtained from larvae left without feeding for three days, and the total fraction of zoohumus obtained from larvae. The amount of basic chemical elements, such as Na, Mg, P, K, Al, Fe, in each sample was determined and the differences between them were assessed. The results showed that the fractional composition of zoohumus has a significant effect on its chemical properties. In particular, high contents of sodium and magnesium were recorded in the composition of untreated samples and samples mixed with feed residues. Based on these analyses, recommendations can be developed to improve the efficiency and standardization of zoohumus as an organic fertilizer in the future.

Introduction

The world population is projected to increase to about 8.1 billion by 2025 and to 9.6 billion by 20509 (Tripathi *et al.,* 2019). According to the Food and Agriculture Organization of the United Nations (FAO), achieving "zero hunger" by 2030 will require increasing food production by 50% compared to 2012 levels (Ikromov *et al.,* 2024) and increasing agricultural productivity by 70% to meet the food demand of the world's growing population by 2050 (Ruzmetova *et al.,* 2024).

These ambitious goals require profound changes in agricultural systems, prioritizing the adoption of sustainable agricultural practices and addressing climate chang (Calicioglu *et al.,* 2019).

A number of negative factors can affect the efficiency of agricultural production. Currently, global agriculture is largely based on the use of chemical fertilizers and pesticides (Calabi *et al.,* 2018). However, their widespread use has negative effects on the environment and human health. Chemical pesticides are associated with an increased risk of immunotoxicity, respiratory diseases, reproductive system changes, hormonal imbalances, and carcinogenicity (Rana *et al.,* 2019). The production of chemical fertilizers, also known as mineral fertilizers, is very energy intensive and accounts for 2% of global greenhouse gas emissions (Beesigamukama *et al.,* 2022). Therefore, new strategies need to be developed to achieve more sustainable agricultural development.

Organic fertilizers such as manure or compost can be a good alternative to chemical fertilizers. They provide plants with smaller amounts of nutrients, but, unlike chemical fertilizers, which are water-soluble and immediately available to plants, they act over a long period of time (Osimani, *et al.,* 2018). Organic fertilizers have important benefits such as soil improvement (microbiological, physicochemical and biochemical), provision of organic matter, increase in the content of available nutrients in the soil or causing less damage to the environment (Shaji *et al.,* 2021). In recent years, extensive research has been carried out in the field of biotechnology and agroecology aimed at obtaining highly effective fertilizers by recycling organic waste. In particular, zoohumus (insect waste) obtained using edible insects, in particular the larvae of *Tenebrio molitor* (yellow mealworm), is gaining great importance as an environmentally friendly, nutrient-rich and biologically active organic fertilizer. Zoohumus is generally rich in a large amount of organic matter, as well as essential nutrients such as nitrogen (N), phosphorus (P) and potassium (K). Zoohumus not only provides plants with essential macronutrients, but also increases the microbial biomass in the soil (Avazova *et al.,* 2024).

Materials and Methods

The studies were conducted in the Biotechnology Laboratory of the Tashkent Institute of Chemical Technology. The sixth generation (F6) of *Tenebrio molitor* (Coleoptera: Tenebrionidae) collected in the southern foothills of Uzbekistan and bred under controlled conditions were used for the experiments. In the study, *Tenebrio molitor* beetles collected in the wild were selected and numbered, and a *Tenebrio molitor* population was created on their basis (Shea, 2005). Dry biomass of wheat bran of standard composition (protein 14-15%, fat 0.8-1.0%) was used to grow *Tenebrio molitor* beetles (Salomova *et al.,* 2025). The cultivation duration for all samples was 28-35 days. Cultivation conditions: a constant temperature of 20-22℃ for keeping insects. A temperature of 10-40℃ was used in the studies. Photoperiodic regime: 8 hours of light, 16 hours of darkness soil (Eshkobilov *et al.,* 2023).

The study was carried out on the samples of zoohumus from *Tenebrio molitor* larvae. The samples were divided into the following five fractions: 1A - purified (fine fraction) zoohumus from larvae; 1B - untreated (coarse fraction) zoohumus from larvae; 2 - purified (1.0 mm sieve) from beetles; 3 - zoohumus from larvae starved for three days; 4 - total fraction of zoohumus from larvae. Each fraction was collected 3 times (n=3) and stored at -20 °C. The samples obtained during the experiment were analyzed for elemental composition using a mass spectrometer. The mass spectrometer was operated under 37 conditions, the capillary temperature was set to 150 °C, the capillary voltage was set to 35 V, and the tube lens voltage was set to 100 V, with a scanning range of m/z 50–500. Conventional methods were used in the studies (Methodical Instructions (MUK 4.1.1482-03).

Results and Discussion

During the research, elemental analysis of various fractions of zoohumus obtained from the feeder insect *Tenebrio molitor* was carried out. The results are presented in Table. During the research, it was noted that zoohumus obtained from the food insect *Tenebrio molitor* can be obtained in the form of different fractions. In particular, zoohumus obtained from larvae comes in two forms: purified (sifted) pure small-sized granular zoohumus and a fraction with small-sized bran grains combined with a mixture of bran, that is, an unpurified zoohumus. If the larvae do not fully consume the food provided, the zoohumus may be of mixed fractions. Therefore, elemental analysis of both fractions was carried out. Also, elemental analysis of zoohumus obtained from beetles and zoohumus isolated from larvae kept without feeding, separately from food, was carried out. Usually, for various studies, larvae are starved for at

least three days, their intestines are cleaned of food, and then they are used.
According to the results of mass spectral analysis, it was noted that the zoohumus obtained in sample 1A contained 329 mg/kg of Na, one of the main chemical elements, and 3000 mg/kg of Mg. In sample 1-B, it was found that the Na element was 510 mg/kg, and the Mg element was 4422 mg/kg. When comparing the results of both samples, it was noted that the Na element was 181 mg/kg higher in sample 1B than in sample 1A. It was also found that the Mg element was 1122 mg/kg higher in sample 1B than in sample 1A.

Analyzing these results, it can be concluded that sample 1A is pure zoohumus produced by larvae, and sample 1B is zoohumus combined with the composition of the nutrient medium not digested by larvae, which led to significant differences in sodium and magnesium retention. In sample 2, it was determined that the amount of Na element was 420 mg/kg, and the amount of Mg element was 6250 mg/kg.

When comparing the samples, it was found that the amount of Na and Mg in sample 2 was 91 mg/kg more than in sample 1A, and the amount of Mg was 3250 mg/kg more than in sample 2. However, it was found that the amount of Na in sample 1-B was 90 mg/kg less than in sample 2, and the amount of Mg was 1828 mg/kg more than in sample 2.

During the research, samples 3 and 4 (3- zoohumus obtained from a larva that had been starved for three days without feeding; 4- total fractional zoohumus obtained from a larva) were also analyzed. It was found that sample 3 contained 1638 mg/kg of Na, and Mg - 3362 mg/kg, while sample 4 contained 527 mg/kg of Na, and Mg - 4732 mg/kg. When comparing the amounts determined according to the results of mass spectral analysis, it was noted that the amount of Na element in sample 3 was 1309 mg/kg higher than sample 1A, 1128 mg/kg higher than sample 1B, and 1218 mg/kg higher than sample 2. When comparing the amount of Mg element with the results of the samples, it was found that it was 362 mg/kg higher than sample 1A, 1060 mg/kg higher than sample 1B, and 2888 mg/kg higher than sample 2. The amount of Na in sample 4 was 158 mg/kg higher than sample 1A, 17 mg/kg higher than sample 1B, and 107 mg/kg higher than sample 2, but on the contrary, it was observed that it was 1111 mg/kg lower than sample 3. The amount of Mg element was recorded as 1732 mg/kg in sample 1-A, 310 mg/kg in sample 1-B, and 1370 mg/kg in sample 3, while it was found to be 1518 mg/kg less in sample 2. According to the results of mass spectral analysis, the amounts of Al, P, and K elements in the zoohumus samples were recorded as follows.

The lowest Al content was recorded in sample 3 (862 mg/kg), which corresponds to the zoohumus obtained from larvae kept without feeding. The highest content was found in sample 4 (3036 mg/kg), which is a sample of the total fraction zoohumus. In general, there is a large difference in the Al content between the samples, which indicates that its origin is mainly associated with feed residues, soil impurities or additional external factors. The highest P content was found in sample 3 (12069 mg/kg), which corresponds to the zoohumus from which the larvae were starved for three days. The lowest P content was found in sample 1A (5857 mg/kg), which is a purified small fraction zoohumus. Also, the element phosphorus is of great importance as a fertilizer for plants, which makes it possible to recommend zoohumus as a fertilizer rich in phosphorus. The highest amount of potassium was found in sample 2 (7054 mg/kg). This sample represents the purified fraction obtained from beetles and sieved. The lowest amount of potassium was recorded in sample 1A (3929 mg/kg). Potassium has a significant impact on the root development and productivity of plants.

Also, according to the results of mass spectral analysis, it was noted that the Ca element was stored in the zoohumus obtained in sample 1-A in an amount of 1786 mg/kg, and the Mn element was stored in an amount of 107 mg/kg. It was determined that the Ca element was stored in sample 1-B in an amount of 2721 mg/kg, and the Mn element in an amount of 145 mg/kg. When comparing the results obtained, it was determined that the Ca element was stored in sample 1-B in an amount of 935 mg/kg, and the Mn element in an amount of 38 mg/kg. It was observed that the Ca element was stored in sample 2 in an amount of 3929 mg/kg, and the Mn element in an amount of 259 mg/kg. When comparing the results of the study, it was found that the Ca element was stored in sample 2 in an amount of 2143 mg/kg compared to sample 1-A, and in sample 1-B in an amount of 1208 mg/kg. It was found that the Mn element was stored in an amount of 152 mg/kg compared to sample 1-A, and in an amount of 114 mg/kg more than sample 1-B. In sample 3, i.e., the zoohumus obtained from a larva that was starved for three days without feeding, the amount of Ca element was found to be 1638 mg/kg, and the Mn element was found to be 68.1 mg/kg. In sample 4, the amount of Ca element was found to be 3214 mg/kg, and the Mn element was found to be 170 mg/kg. When the results were compared, it was noted that the amount of Ca element was 148 mg/kg compared to sample 1-A, 1083 mg/kg compared to sample 1-B, and in a large amount, it was found to be 2291 mg/kg less than sample 2. The amount of Mn element was found to be 38.9 mg/kg higher than sample 1-A, 76.9 mg/kg higher than sample 1-B, and 190.9 mg/kg higher than sample 2. In sample 4, the amount of Ca element was found to be 1428 mg/kg lower than sample 1-A, 493 mg/kg lower than sample 1-B, and 1576 mg/kg lower than sample 3. It was found to be 715 mg/kg higher than sample 2. The amount of Mn element was found to be 63 mg/kg higher than sample 1-A, 25 mg/kg higher than sample 1-B, and 101.9 mg/kg higher than sample 3, but it was found to be 89 mg/kg lower than sample 2. The element iron (Fe) in zoohumus is one of the important microelements for plants, which participates in chlorophyll synthesis, oxidation-reduction reactions, and enzymatic activity. Based on the conducted analyses, the amount of Fe in zoohumus was determined as follows. It was noted that it was 450 mg/kg in sample 1-A, 680 mg/kg in sample 1-B, 589 mg/kg in sample 2, 293 mg/kg in sample 3, and 884 mg/kg in sample 4. When comparing the analyses obtained during the studies, it was observed that the amount of Fe in sample 1-A was 230 mg/kg less than in sample 1B, 139 mg/kg less than in sample 2, and 434 mg/kg less than in sample 4, while it was noted that it was 157 mg/kg more than in sample 3. At the same time, very low amounts of heavy metals such as Cd, Pb, As, Ag were detected in the samples, and the analysis revealed that their concentration was below the safety standard, and such zoohumus is considered environmentally safe. During the research, it was found that microelements such as Ni, Cu, Zn, Mo were also found in the composition of various fractional zoohumus obtained on the basis of the food insect *Tenebrio molitor*, and elemental analysis of the following elements was also carried out.

The Ni element was found to be 1.86 mg/kg in sample 1-A, 1.62 mg/kg in sample 1-B, 1.70 mg/kg in sample 2, 2.16 mg/kg in sample 3, and 5.27 mg/kg in sample 4, while the Cu element was found to be 11.4 mg/kg in sample 1A, 14.5 mg/kg in sample 1-B, 17.9 mg/kg in sample 2, 32.8 mg/kg in sample 3, and 14.3 mg/kg in sample 4. When the results were compared, it was found that the Ni element in sample 1A was 0.24 mg/kg higher than in sample 1B and 0.16 mg/kg higher than in sample 2. It was observed that it was 0.3 mg/kg lower than in sample 3 and 3.41 mg/kg lower than in sample 4. When comparing the results of the Cu element, it was found that sample 1A contained 3.1 mg/kg less than sample 1B, 6.5 mg/kg less than sample 2, 21.4 mg/kg less than sample 3, and 2.9 mg/kg less than sample 4. The lowest amount of Zn element was observed in sample 1A, i.e. 57.1 mg/kg, and the highest amount was observed in sample 2, i.e. 152 mg/kg. The highest amount of Mo element was detected in the total fractional zoohumus obtained from the larva of sample 4. It can be seen that the amount of Mo element in this fraction is several times higher than in other fractions. That is, it was recorded as 33.13 mg/kg higher than in sample 1A, 33.32 mg/kg higher than in sample 1B, 32.49 mg/kg higher than in sample 2, and 32.77 mg/kg higher than in sample 3.

In this study, the content of chemical elements in different fractions of zoohumus obtained from *Tenebrio molitor* larvae and beetles (1A - purified (small fraction) zoohumus obtained from larvae; 1B - untreated (large fraction) zoohumus obtained from larvae; 2 - purified (1.0 mm sieve) obtained from beetles; 3 - zoohumus obtained from larvae starved for three days; 4 - total fraction zoohumus obtained from larvae) was studied by mass spectral analysis. The results obtained showed that the content of elements in zoohumus is directly related to the conditions of its formation, the degree of purification and the state of mixing with food. As a result of the studies, it was found that the content of chemical elements in zoohumus obtained from the food insect *Tenebrio molitor* differs significantly depending on the different fractions. According to mass spectral analysis, it was observed that the concentrations of major elements, especially Na and Mg, varied between samples.

In particular, the purified small-fraction zoohumus sample (1A) contained the lowest amounts of Na (329 mg/kg) and Mg (3000 mg/kg), while the untreated (large-fraction) zoohumus sample (1B) contained significantly more of these elements - 510 mg/kg Na and 4422 mg/kg Mg, respectively.

This may be an indication of the presence of incompletely decomposed feed residues in sample 1B. Also, the purified zoohumus obtained from beetles (sample 2) contained the highest Mg content (6250 mg/kg), while the Na content in this sample was 420 mg/kg. In the zoohumus obtained from starved larvae (sample 3), the highest amount of Na was detected - 1638 mg/kg, which indicates that starvation leads to complete elimination of nutrients from the intestine and accumulation of elements.

Table.1 Elemental analysis of zoohumus obtained from *Tenebrio molitor*

№	Lab №	Options №	Li	Be	B *	Na *	Mg *	Al *	P *	K *	Ca *	Sc	Ti *	V	Cr	Mn	Fe *	Co
	Measuring range of specific elements		**0,05-4000**	**0,05-4000**	**0,10-4000**	**0,004-11%**	**0,004-11%**	**0,002-20%**		**0,008-30%**	**0,005-28%**	**0,10-4000**	**0,000 6-9%**	**0,10-4000**	**1,0-4000**	**0,002-10%**	**0,006-30%**	**0,10-4000**
1	1-1	1a	1,29	0,024	2,36	329	3000	1000	5857	3929	1786	0,35	34,3	1,71	2,29	107	450	0,286
2	1-2	1b	1,19	0,036	3,06	510	4422	1701	8418	5187	2721	0,37	46,8	2,64	3,40	145	680	0,349
3	1-3	2	0,893	0,020	4,38	420	6250	1339	10714	7054	3929	0,32	53,6	2,86	4,02	259	589	0,304
4	1-4	3	0,690	0,020	4,91	1638	3362	862	12069	6983	1638	0,25	42,2	3,79	6,12	68,1	293	0,310
5	1-5	4	0,982	0,045	4,02	527	4732	3036	8929	6071	3214	0,49	75,9	4,02	5,89	170	884	0,384
№	**Lab №**	**Options №**	**Ni**	**Cu**	**Zn**	**Ga**	**As**	**Se**	**Rb**	**Sr**	**Y**	**Zr ***	**Nb**	**Mo**	**Ag**	**Cd**	**In***	
	Measuring range of specific elements		**1,0-4000**	**1,0-4000**	**1,0-4000**	**0,10-4000**	**0,10-4000**	**0,50-4000**	**0,10-4000**	**0,10-4000**	**0,10-4000**		**0,005-4000**	**0,10-4000**	**0,05-10,0**	**0,005-4000**		
1	1-1	1a	1,86	11,4	57,1	0,321	0,543	<0,50	4,36	18,6	0,271	0,429	0,100	2,57	19,3	0,007	<0,005	
2	1-2	1b	1,62	14,5	74,0	0,374	0,808	0,561	5,78	27,2	0,417	0,468	0,136	2,38	15,3	0,011	<0,005	
3	1-3	2	1,70	17,9	152	0,429	0,893	0,563	6,88	33,9	0,366	0,893	0,116	3,21	0,179	0,016	<0,005	
4	1-4	3	2,16	32,8	129	0,310	0,948	<0,50	4,48	30,2	0,276	0,353	0,061	2,93	36,2	0,009	<0,005	
5	1-5	4	5,27	14,3	81,3	0,446	1,43	0,563	8,84	31,3	0,705	1,34	0,277	35,7	6,70	0,017	<0,005	
№	**Lab №**	**Options №**	**Sn**	**Sb**	**Te**	**Cs**	**Ba**	**La**	**Ce**	**Pr**	**Nd**	**Sm**	**Eu**	**Gd**	**Tb**	**Dy**	**Ho**	
	Measuring range of specific elements		**0,10-10**	**0,10-4000**	**0,30-4000**	**0,02-4000**	**0,10-4000**	**0,50-4000**	**0,04-4000**	**0,01-4000**	**0,01-4000**	**0,01-4000**	**0,01-4000**	**0,01-4000**	**0,01-4000**	**0,01-4000**	**0,01-4000**	
1	1-1	1a	0,061	0,062	<0,30	0,114	18,57	0,450	0,857	0,100	0,336	0,062	0,016	0,069	0,009	0,034	0,009	
2	1-2	1b	0,084	0,119	<0,30	0,145	20,41	0,655	1,36	0,145	0,604	0,128	0,023	0,085	0,012	0,085	0,015	
3	1-3	2	0,107	0,116	<0,30	0,107	26,79	0,625	0,982	0,161	0,563	0,116	0,030	0,116	0,012	0,067	0,012	
4	1-4	3	0,060	0,112	<0,30	0,075	20,69	0,491	0,681	0,129	0,474	0,077	0,016	0,081	0,009	0,059	0,009	
5	1-5	4	0,089	0,223	<0,30	0,214	25,89	1,16	2,05	0,295	1,07	0,170	0,047	0,170	0,022	0,152	0,023	

№	Lab №	Options №	Er	Tm	Yb	Lu	Hf	Ta	W	Re	Pt*	Au *	Tl	Pb	Bi	Th	U	
	Measuring range of specific elements		**0,01-4000**	**0,01-4000**	**0,01-4000**	**0,01-4000**	**0,05-4000**	**0,04-4000**	**0,08-4000**	**0,01-4000**	**0,05-4000**	**0,05-4000**	**0,01-4000**	**0,1-4000**	**0,01-4000**	**0,01-4000**	**0,01-4000**	
1	1-1	1a	0,022	0,003	0,024	0,003	0,015	0,004	0,143	<0,01	<0,05	<0,05	0,067	1,00	0,009	0,129	0,079	
2	1-2	1b	0,040	0,006	0,048	0,005	0,019	0,005	0,077	<0,01	<0,05	<0,05	0,085	1,45	0,009	0,213	0,153	
3	1-3	2	0,030	0,004	0,040	0,004	0,028	0,013	0,098	<0,01	<0,05	<0,05	0,076	2,23	0,011	0,179	0,179	
4	1-4	3	0,022	0,004	0,027	0,004	0,015	0,005	0,064	<0,01	<0,05	<0,05	0,066	1,64	0,007	0,103	0,276	
5	1-5	4	0,056	0,010	0,068	0,014	0,045	0,021	1,16	<0,01	<0,05	<0,05	0,063	1,61	0,013	0,402	1,696	

Note: 1A - purified (fine fraction) zoohumus obtained from the larva; 1B - unprocessed (coarse fraction) zoohumus obtained from the larva; 2 - purified (passed through a 1.0 mm sieve) obtained from the beetle; 3 - zoohumus obtained from the larva that had starved for three days without feeding; 4 - total fraction of zoohumus obtained from the larva.

In the general fractional zoohumus (sample 4), Mg (4732 mg/kg) was recorded in high amounts, and Na (527 mg/kg) in moderate amounts. This fraction showed average indicators when compared with other samples in terms of the amount of elements.

In general, the analyses showed that the fractional differences in the composition of zoohumus depend on the factors forming it (the nutritional status of the larva, the purity of the feed, the degree of sieving).

The results obtained justify the need to take into account the chemical composition of zoohumus when using it as a fertilizer. In addition, the purified fractions have more accurate and stable element amounts and can be considered a preferable option in research and practice.

Author Contributions

S. S. Salomova: Investigation, formal analysis, writing—original draft. N. A. Khujamshukurov: Validation, methodology, writing—reviewing. X. N. Isabaev:—Formal analysis, writing—review and editing. D. B. Turabekova: Investigation, writing—reviewing. D. Kh. Kuchkarova: Resources, investigation writing—reviewing. Alvina Farooqui: Validation, formal analysis, writing—reviewing. Tripath Gyanendra: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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