Research Article

The potentiality of toxic metals to wheat crop as affected by municipal solid waste: An implication to human health

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Abstract

The by-product of industrial and municipal wastewater is sewage sludge. It is enriched with a lot of organic nutrients. The sewage sludge that has high amount of organic matter, micronutrients, and macro-nutrients, are usually used as fertilizer for horticultural crops. The impact of sewage sludge on heavy metal's uptake ratio by wheat variety is observed by conducting a pot experiment. The amount of heavy metals in various parts of wheat plant was analyzed by atomic absorption spectrophotometer (AA-6300 Shimadzu Japan). The metals concentration in grains of wheat ranged from 0.64 to 0.97 for Zn, 0.76 to 0.93 for Co, 1.30 to 1.61 for Cd and 0.81 to 1.58 mg/kg for Fe, respectively. All metal concentrations were found below the permissible limits suggested by FAO/WHO. The calculation for translocation and bio-concentration factor for various parts of the wheat crop was done. However, there may be a risk of the presence of potentially unsafe substances in the sewage sludge e.g., pathogens and heavy metals.

Keywords: Contamination; Health risk; Metal; Sewage sludge; *Triticum aestivum*

Introduction

Sewage sludge is considered as a good biological resource for recycling the nutrients when it is applied in the agricultural land. Thus, sewage sludge has an important role in sustainable agriculture. Sewage sludge gives a better plant yield when it is used in agricultural land as an organic fertilizer. When sewage sludge was used as organic fertiliser, it produces a good plant yield [1-3].

For burgeoning population wheat (*Triticum aestivum* L.) is considered as a potential food source. Every year, wheat is grown in millions of tons [4]. In Pakistan, wheat has central position in cereals, and it has a 66% annual food crop area, it also provides average diet calories about 60% [5]. Different factors effects on wheat, such as heavy metals, infertile soil, drought, waterlogging, and salinity. As a

result of these factors wheat yields becomes low [6, 7].

In the food chain, the role of heavy metal is focused by the sewage sludge research application. Microbial activity in soil increased due to sewage sludge application at recommended rates and tied up to the unavailability of the heavy metals to the plant and soil [8, 9].

The sewage sludge use in bio-solids form is considered as the pervasiveness of microorganisms and it is another factor which needs careful attention. The biosolids sewage sludge contains viruses, pathogenic bacteria, protozoa, and parasitic helminths, etc. These pathogens cause potential health hazards to human, plants, and animals. Microbes cause health risk when sewage sludge applied to land. Among identified microbes *Taenia* and *Salmonella* were the greatest concern. By appropriate sludge treatment, there would be a maximum reduction in several parasitic and pathogenic organisms prior to its application into land. Furthermore, after its application, health hazards become reduced because of some factors like climate, time and soil microorganisms [10].

The development and growth of plants may be inhibited by those heavy metals which easily assimilated by plants at high concentrations. Moreover, metals cause damage to cell membranes in plants, metals decreases the transpiration rate, damaging the photosynthetic organelles, increasing the lipid peroxidation, and destroying protein synthesis. Most of the metals including Ni, Cr, Cd, Cu, Pb, Zn are potentially called toxic elements that originate in sewage. The association of these metals with settable solids is concentrated in sludge in the course of primary treatment as well as secondary treatment processes [11].

The food chain is affected by heavy metals acquisition in plants and threatens the health of humans. Environmental stress is very effective on plant protection mechanisms, as plants have ability to accumulate the proline [12].

Heavy metals have a high environmental concern, harmful to animals, humans and also susceptible to food chain. In urban areas heavy metals come from different sources. In topsoil's heavy metals contamination arises due to atmospheric pollution [13-15].

The present research was performed with objectives: (1) to examine the impact of sewage sludge on uptake of metal by different portions of wheat plant, (2) to determine transfer factor and factor of bioconcentration, (3) to appraise pollution severity of soil, (4) to estimate health risk index.

Materials and Methods
Experimental design

In Botany Department's ground, a pot experiment was conducted in University of Sargodha from Sargodha during the year 2016-2017. The soil used in the present study was collected from ponds and ditches located in nearby areas of Sargodha. Took 12 pots and filled with soil. After that, wheat variety (Aas) seeds were sown in four cultivation groups with the amendment rates of sewage sludge: T-I consisted of 100% ground soil, T-II consisted of 75% ground soil and 25% sludge of sewage, T-III 50% ground soil and 50% sludge of sewage, T-IV consisted of 25% ground soil and 75% sludge of sewage.

Filling of each pot with respective treatment in 3 kg was done. After filling pots were placed as randomized complete block (RCB) design and three replications per treatment was made. Then by using groundwater, all the pots were irrigated. Irrigation was done two times in a week and in each pot 12 seeds were sown. Twelve seeds were hand sown on November 2016 in each pot. After that Seed germination data was noted and finally for complete plant growth, five plants were contained in every pot. Maturation period was five months.

Sample collection and preparation

In April 2017, harvesting was done. From each pot, all samples were collected after harvesting, and then for two days the drying of plant material was carried out at 70 °C, was weighed and ground into a fine powder.

Sample digestion

Samples digestion was brought about in a 3: 1 HNO_3 mixture: H_2O_2 by the wet digestion method. All the samples were kept in a measuring flask up to 50 ml as final volume. The samples filtration was then carried out by Whatman filter paper No. 42. The samples of soil were assembled from the upper 3-5 cm layer of the soil from every pot. After air drying, samples of soil were placed in the oven for two days at 65 °C. The samples were digested in the same manner.

Heavy metal analysis

Atomic absorption spectrophotometer (AA-6300 Shimadzu Japan) was used for the analysis of metals. Zn, Co, Fe, and Cd concentration had been determined by atomic absorption spectrophotometer respectively.

Quality control

To assess the reliability and assurance of the data, such measures were taken. By comparing with the international standards, the precision of the results can be done. The repeated analyses of the examined samples can also verify the accuracy of the analyses.

Data analysis

Data analysis was done through SPSS (ver. 14.0, SPSS Inc., Chicago, IL) and firstly, one-way analysis of variance (ANOVA) was used for data analysis. Then R-value (coefficient of correlation) and mean standard deviation of crop and soil parameters having concentrations of sludge were calculated by using MS Excel (ver. 2003, Microsoft Redmond Campus, Redmond, WA) and finally graphs were made through Sigma Plot (ver. 12.3, Systat Software, Inc., Chicago, IL).

For heavy metals accumulation from the soil, a \bioaccumulation (BAF) factor was measured in to check the plants' efficiency [16]:

BAF = Concentration of metal in roots (mg/kg) / Concentration of metal in soil (mg/kg)

For translocation of heavy metal from roots towards the shoot of plant, a translocation factor (TF) found out to check the capability of plants.

TF = Concentration of metal in shoots (mg/kg) / Concentration of metal in roots (mg/kg)

Pollution load index (PLI) of every treatment was calculated [17].

PLI = Determined level of metal in the soil being investigated ∕ reference value of soil metals

Daily intake of metal (DIM) was computed according to Sajjad *et al*. [18].

$DIM = D_{food\ intake} \times C_{metal} / B_{average\ weight}$

Where: C_{metal}, $B_{average\ weight}$, and $D_{food\ intake}$ refer to concentrations of heavy metal (mg/kg) in food crop, average body weight (kg) and daily intake of a food crop (kg/day) respectively.

Body's average weight was taken as 55.9 kg and metal's daily intake in wheat as 0.318 kg/day per person respectively.

According to Cui *et al*. [19], health risk index (HRI) was described in terms of estimated exposure relation to metal by oral reference dose and food crop.

HRI (Health risk index) = DIM/ R_fD [20]

Oral reference dose (R_fD) is the maximum acceptable oral dose of heavy metals.

As described in USEPA [20], HRI >1 is considered as very dangerous for humans.

For calculating soil pollution extent, enrichment factor (EF) was calculated the following and Al-Hwaiti and Al-Khashman [21].

EF = Metal concentration examined in amended soil/ Metal's concentration in control soil.

Results and discussion

Zinc concentration in the soil

Analysis of variance of Zn concentration showed a significant effect ($p<0.05$) in soil from four treatments which were employed for growing wheat variety. The

Zn value detected in the soil was 2.61 to 2.74 mg/kg in the present study. The mean Zn concentration values were in order: T-IV>T-III>T-II>T-I (Table 1).

Ozyazici [22] obtained the highest value i.e. 83.38-58.81 (mg/kg) for zinc as compared to recent research values. Similarly, Yu et al. [23] founded the greater value of Zn than the value attained in current work. Stefanovic et al. [24] observed a higher amount of Zn than present, by working on different varieties of wheat. Current Zn values are lower than the calculations of Ran et al. [25]. Zinc is a vital micronutrient and is involved in numerous metabolic processes of plants.

Table 1. Metal's mean concentration (mg/kg) in soils, shoots, roots and grains of wheat

Metal	T-I	T-II	T-III	T-IV
Soil				
Zn	2.61±0.13	2.64±0.14	2.68±0.15	2.74±0.16
Co	1.06±0.03	1.16±0.04	1.19±0.05	1.22±0.06
Cd	3.34±0.13	3.38±0.15	3.46±0.17	3.44±0.19
Fe	32.72±1.92	39.07±1.99	44.23±2.21	44.25±2.19
Root				
Zn	2.16±0.11	2.19±0.13	2.23±0.14	2.31±0.15
Co	1.05±0.04	1.09±0.05	1.12±0.06	1.21±0.07
Cd	2.89±0.11	2.92±0.12	3.11±0.19	2.99±0.13
Fe	29.73±1.91	36.07±2.04	41.23±2.21	41.24±2.22
Shoot				
Zn	1.92±0.08	1.93±0.09	1.98±0.11	2.05±0.12
Co	0.86±0.03	0.97±0.04	0.99±0.05	1.03±0.06
Cd	2.64±0.11	2.68±0.12	2.81±0.15	2.74±0.17
Fe	25.23±2.01	31.57±2.11	36.73±2.09	36.75±2.08
Grains				
Zn	0.64±0.09	0.89±0.11	0.97±0.12	0.97±0.12
Co	0.76±0.03	0.87±0.04	0.89±0.05	0.93±0.06
Cd	1.39±0.09	1.61±0.13	1.49±0.11	1.52±0.14
Fe	0.81±0.28	1.32±0.34	1.42±0.36	1.58±0.33

The concentration of zinc in wheat plant

Zn concentration in root was observed to be between 2.16 to 2.31 mg/kg in our findings of research. The order was: T-IV>T-III>T-II>T-I. Zn concentration in the shoot was observed between 1.92 to 2.05 mg/kg. The highest value found in T-IV and the lowest value found in T-I. Zinc concentration in grain varied from 0.64 to 0.97 mg/kg. The Zn mean concentration values were in order: T-IV=T-III>T-II>T-I. The lowest value was found in T-I and the highest value was found in T-IV (Table 1).

Antolin et al. [26] stated that grain yield is increased with sewage sludge application because the amended soil's microbiological properties had improved, due to which nutrients recycling for the crop is promoted. In normal plants, the mean concentration of Zn in aerial tissues was 66 mg/kg [27].

The concentration of cobalt in soil

Analysis of variance showed non-significant effect ($p>0.05$) on Co concentration which was taken from 4 treatments in the soil which were employed for the growth of wheat variety. The current finding of Co in the soil was 1.06 to 1.22 mg/kg. The lowest value found in T-I and the highest value found in T-IV. The order was: T-IV>T-III>T-II>T-I (Table 1).

Cobalt is present in the ecosystem in various forms. Ahmad et al. [28] obtained its lower value as compared to present value. In trace amount Co is essential for animals and plants but toxic at high

concentration [29]. Cobalt toxicity depends upon nature of soil. Toxicity of Co will be greater in the presence of acidic soil [30].

The concentration of cobalt in wheat plant

The Co value in the recent study ranged between 1.05-1.21 mg/kg in the root. The mean Co concentration was found in following order: T-IV>T-II>T-III>T-I. The range of Co concentration in shoot was 0.86-1.03 mg/kg. At T-I the mean concentration value of Co was 0.86 mg/kg and gradually increase towards T-IV. Co concentration in grain varied from 0.76-0.93 mg/kg. The mean concentration values of Co were in order: T-IV>T-III>T-II>T-I. The lowest value was observed in T-I and the highest value was observed in T-IV (Table 1).

As compared to other metals Co toxicity was low. Its toxicity depends upon plant species, nature of soil and composition of the soil. Toxicity of Co affects humans through contamination of food chain [31]. It also plays important role in nitrogen fixation of leguminous plants and has detrimental effects at high concentrations in plants [32].

The concentration of cadmium in soil

Variance analysis of Cd concentration showed a significant effect ($p<0.05$) in soil of four treatments which are employed for the growth of wheat variety. In this study, value of Cd was found to be varied from 3.34-3.44 mg/kg in the soil samples (Table 1).

Cadmium value attained by Ozyazici [22] was smaller as compared with the value of the present study. Less value of Cd (0.46 mg/kg) was reported by Yu *et al*. [23] in distinction with existing Cd value. Maximum permissible value for Cd was 1-3 mg/kg according to EU standards reported by McGrath *et al*. [33]. The current value of Cd lies under permissible limits so it was safe. Cadmium entered in soil by the application of phosphate fertilizers, because it was added in fertilizers as impurity. Cadmium

transferred quickly from root to edible parts as compared to other metals [34]. It may be hazardous for the population of microorganisms [35].

The concentration of cadmium in wheat plant

The highest value was found in T-IV. The Cd value showed variation in the range of 2.89 to 3.11 mg/kg in wheat root. Cadmium concentration in shoot was found in range of 2.64 to 2.81 mg/kg. At T-I the Cd mean concentration value was 2.64 mg/kg and highest value was 2.81 mg/kg at T-III. Cd concentration in grain varied 1.39 to 1.61 mg/kg. The mean values of Cd were in order: T-II>T-IV>T-III>T-I. The lowest value was observed in T-I and the highest value was observed at T-II (Table 1).

Stefanovic *et al*. [24] obtained the lesser value of Cd (0.01 to 0.21 mg/kg) in distinction with the present Cd values. The reason for high concentration of Cd may be the total Cd of soil and pH. These two factors may be responsible for the increased concentration of Cd in wheat grains. Organic matter, pH and soil Cd is significantly influenced by the application of metal salts and sewage sludge [36]. Plant uptake Cd through their roots and accumulate it inedible parts. It accumulates in fat tissues and milk of animals [37].

The concentration of iron in soil

Analysis of variance of Fe concentration showed a significant effect ($p<0.05$) in the soil of 4 treatments employed for the growth of wheat variety Aas. Iron concentration in soil varied from 32.72 to 44.25 mg/kg (Table 1).

Stefanovic *et al*. [24] attained the highest value of Fe as compared to current value when he analyzed different varieties of wheat and these values varied from 29.46-69.26 mg/kg. According to FAO/WHO [38] maximum permissible level of Fe for crops was 425.50 mg/kg and current value lies under this safe limit. Iron in plants is slightly immobile. Iron forms stable structures and this might be the reason for high Fe concentration in soil in which

wheat variety (Aas) was grown and irrigated by altered doses of domestic wastewater and groundwater.

The concentration of iron in wheat plant
Analysis of variance of Fe concentration showed a significant effect ($p<0.05$) in root of 4 treatments employed for the growth of wheat variety. Iron concentration in root varied from 29.73 to 41.24 mg/kg. Variance analysis of Fe concentration showed significant effect ($p<0.05$) in shoot from four treatments used for growing wheat variety. Iron concentration in shoot varied from 25.23 to 36.75 mg/kg. Analysis of variance of Fe concentration showed significant effect i.e. $p<0.05$ in grain of 4 treatments employed for the growth of wheat variety. Iron concentration in grain shows variation from 0.81 to 1.58 mg/kg (Table 1).

Value of Fe obtained from present work was lesser than those found by Stefanovic et al. [24]. The high concentration of Fe was found in plants treated with sewage sludge [39]. Iron is essential for the growth of plants and animals. Many plants and animal proteins contain Fe that is necessary for the proper functioning of proteins and enzymes. It is confirmed from results that heavy metal concentration of

soil increased with the increase in sewage sludge concentration. The high concentration of Fe in soil may be due to the abundance of Iron in earth crust [40].

Correlation
In Fe soil-root and root-shoot, the results gave positive significant correlation and shoot-grains gave positive correlation. In soil-root and root-shoot Co gave positive correlation and shoot-grain gave positive significant correlation. In Cd soil-root and shoot-grains demonstrated positive correlation and root-shoot demonstrated positive significant correlation. And Fe soil-root, root-shoot and shoot-grains in all showed positive significant correlation (Table 2).

Herren and Feller [41] and Hart et al. [42] indicated that Cd was accumulated in grains because it translocates by the help of vascular tissues and significant results were obtained. When Cd translocation occurs from stem and/or leaves to the grains, it stores in wheat grains and its current correlation values were in contrast with those obtained by Ashfaq et al. [43]. It was founded that availability of metals to plant was strongly controlled by metal's solubility in acid, chemical form and reducible form of metal [44].

Table 2. Correlation between soil-root, root-shoot and shoot-grain metal concentrations of wheat

Metal	Correlation		
	Soil-Root	**Root-Shoot**	**Shoot-Grains**
Zn	.997**	.992**	.707
Co	.873	.878	1.000**
Cd	.919	.988*	.195
Fe	1.000**	1.000**	.961*

Bioconcentration factor
At T-I the lowest value of Zn was 0.827. And at T-IV the highest value of Mg was 0.843. The bioconcentration factor of Co in the wheat grains varied from 0.939 to 0.9918. At T-I the BCF of Cd in the wheat grains of Aas was 0.865. At T-II the BCF of Cd was 0.863 mg/kg. And T-III and T-IV the values of Cd were 0.898 and 0.869. The bioconcentration factor of Fe in the

wheat grains varied from 0.908 to 0.9321 (Table 3).

Recent BCF values were higher as compared to those found by Puschenreiter et al. [45]. The higher value of Cd was reported in current research work than those with the results attained by Wang et al. [46]. The bioconcentration factor values for Cd were >1 as observed by Iqbal et al. [47]. According to Ran et al.

[25], the BCF value for Cd and Zn was low in distinction with the present research work.

Transfer factor

The values of TF at all treatments were found in the following order: Co> Cd> Zn> Fe. The TF for Co was highest while Fe was lowest among all treatments (Table 3).

All values for transfer ratios of Fe, Cd, Co and Zn in wheat were less than 1. For each heavy metal, both stem and leaf showed higher translocation ratio in wheat as compared to wheat grain. Furthermore, the translocation factor (TF) for leaf and stem to grain (HM grain/ HM stem and leaf) was reported to be lesser as compared to those of root to stem/leaf of wheat. The present research is in food agreement with the findings found in wheat by Bose and Battacharya [48] when the wheat was grown in soil fertilized with sewage sludge.

Table 3. Bioconcentration factor and transfer factor for all metals of wheat

Metal	Treatment			
	T-I	T-II	T-III	T-IV
BCF				
Zn	0.8275	0.8295	0.8320	0.8430
Co	0.9905	0.9396	0.9411	0.9918
Cd	0.8652	0.8639	0.8988	0.8691
Fe	0.9086	0.9232	0.9321	0.9319
TF				
Zn	0.2962	0.4063	0.4349	0.4199
Co	0.7238	0.7981	0.7946	0.7685
Cd	0.4809	0.5513	0.4791	0.5083
Fe	0.0272	0.0365	0.0344	0.0383

Pollution load index

The values of PLI at T-I, T-II, T-III and T-IV were present in the following sequence: Cd> Fe> Co> Zn. The values of PLI for Cd were greatest but for Zn were lowest between all treatments (Table 4).

Results show that PLI value was lower for Zn but higher for Cd. The pollution load index estimated how many times the concentration of metal goes beyond the background concentration and presents the toxicity level of metals in particular samples. Pollution load index values studied by Rabee [49] were reported to be varied from 0.301-0.970 and were less than one (<1). He also observed that these values correspond with present values of PLI except Cd. Ahmad *et al*. [28] observed higher PLI values in contrast to the existing values.

Enrichment factor

The values of EF at T-I were present in the following order: Cd> Co> Zn> Fe, at T-II and T-III and T-IV was: Cd> Zn> Co> Fe (Table 4).

Ghrefat *et al.* [50] reported EF > 1 recommends that metals come from anthropogenic sources. According to the observation of McCave [51] and Horowitz and Elrick [52], EF value was high due to ionic attraction and surface adsorption. Ra *et al.* [53] noticed greater values of EF for Co and Cd while, lower Zn value in distinction with recent research.

Daily intake of metals

The daily intake of metal at T-I, T-II and T-III was observed at the order: Cd> Fe> Co> Zn, and at T-IV was: Fe> Cd> Zn> Co (Table 5).

Recent values of DIM were less for Zn and Cd in comparison with obtained results of Gupta *et al*. [54]. In humans, metal's toxicity level is dependent on its daily intake. Human health is badly affected when it is encountered with daily consumption of contaminated food. According to Sajjad *et al*. [18] country's

population can be at high risk in future if DIM equal or greater than 1. For all metals, current obtained DIM values were less than 1. It was reported by Huang *et al.* [55] that wheat is consumed as 30% of their staple food by the people of Jiangsu province. Daily intake of metals values for rural and urban adults were 102 g and 62.5 g respectively. Values for daily intake of wheat for urban and rural children upto 6 years old were reported as 20.8 g and 34.1 g per day respectively.

Health risk index

Health risk index at T-I, T-II, T-III and T-IV was observed in this sequence: Cd> Co> Zn> Fe. The values of HRI were highest of Cd and lowest for Fe in all treatments (Table 5).

Result describes that HRI was lower for Fe and Zn but for Cd, it was higher in all treatments. Fe and Co showed HRI values in acceptable range, while for Cd obtained values were not safe i.e. more than 1. Khan *et al.* [56] found heavy metals having HRI values <1 is considered to be safe and did not hinder the food chain. Karak and Bhattacharyya [57] reported same value for Zn as obtained in recent research but lower value for Cd. Value of HRI is highly dependent on the and R_fD and metal's daily intake. R_fD is the estimated value for metals per day that has no deleterious effects on human body. R_fD values for Cd and Zn were 0.001 and 0.30 mg/kg correspondingly [58].

Table 4. Pollution load index and enrichment factor of wheat

Metal	Treatment			
	T-I	T-II	T-III	T-IV
PLI				
Zn	0.0590	0.0597	0.0606	0.0620
Co	0.1164	0.1274	0.1307	0.1340
Cd	2.2416	2.2684	2.3221	2.3087
Fe	0.5750	0.6866	0.7773	0.7776
EF				
Zn	0.1090	0.1498	0.1609	0.1573
Co	0.1304	0.1365	0.1361	0.1387
Cd	3.1004	3.5486	3.2082	3.2918
Fe	0.0033	0.0045	0.0042	0.0047

Table 5. Daily intake of metal and health risk index via intake of wheat

Metal	Treatment			
	T-I	T-II	T-III	T-IV
DIM				
Zn	0.0027	0.0038	0.0041	0.0041
Co	0.0032	0.0037	0.0038	0.0040
Cd	0.0060	0.0069	0.0064	0.0065
Fe	0.0035	0.0057	0.0061	0.0068
HRI				
Zn	0.0092	0.0128	0.0139	0.0139
Co	0.0765	0.0875	0.0896	0.0936
Cd	6.018	6.97	6.45	6.58
Fe	0.0050	0.0081	0.0087	0.0097

Conclusion

By keeping in view the above review, it can be concluded that sewage sludge plays a significant role in sustainable agriculture

when used as a soil conditioner/organic fertilizer. It increases the macro and micro-nutrients of the soil. It also improves its organic matter contents that result in

improvement of soil physical condition, as well as its biological activity and health. Sewage sludge's application on wheat crop gives positive responses. Organic matter present in the sewage sludge binds the contaminants (pollutants and heavy metals) and ultimately results in a reduction of negative environmental effects of these contaminants. In wheat variety (Aas), the concentrations of Co, Cd, Zn, and Fe were in the acceptable limit of standards of WHO/FAO. Consequently, this study suggests that if sewage sludge is used in smaller ratio in soil as soil conditioner/ fertilizer then there will be no major danger because of heavy metals present in the sewage sludge. Therefore, current study recommends using the sludge of sewage as a biological resource for conditioning of soil and contamination of both wheat and soil can also be avoided with the use of lower amount of sewage sludge.

Authors' contributions

Conceived and designed the experiments: ZI Khan & K Ahmad, Performed the experiments: F Zameer, M Akhter & P Akhtar, Analyzed the data: K Wajid, M Ghazzal & A Ashfaq, Contributed reagents/ materials/ analysis tools: H Bahir N Mehmood & M Nadeem, Wrote the paper: AI Batool, IS Malik & M Munir.

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