## RESEARCH



# A preliminary study on heavy metal monitoring in soil and guar (*Cyamopsis tetragonoloba*) biomass amended with sewage sludge

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Abstract Applying sewage sludge in agricultural soils is an interesting source of organic matter. This study aimed to monitor concentrations of heavy elements in soil and guar plants, which can pose a risk to the health of humans and animals if they enter the food chain through the soil-plant system. The experiment revealed that applying sludge increased the amount of organic matter, total nitrogen, potassium, and phosphorus in the soil. Additionally, the concentration of heavy metals such as Pb, Co, Cr, Ni, Cu, and Zn in all treatments remained below the permissible limits for soil. The highest plant height and plant dry weight were recorded in the sludge and sludge + fertilizer treatments. The dry weight of the guar varied from 629 g m<sup>-1</sup> in the control treatment to 1050 g  $m^{-1}$  in the sludge + fertilizer plots. The use of sludge increased the accumulation of heavy metals in the above-ground parts of the guar plant compared to the control. However, the level of heavy metal remained within the normal range and below the toxic concentration. Our results also showed that the application of sludge along with fertilizer improved the quality of the guar forage by increasing the levels of crude

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protein, digestible dry matter and water-soluble carbohydrates. Overall, the results indicated that using sludge as organic fertilizer can improve soil properties, reduce the use of inorganic fertilizers, and decrease the harmful effects of heavy metals on the environment and health in the research area.

**Keywords** Environmental hazards · Nutrient availability · Organic fertilizer · Soil heavy metal monitoring · Sustainable development

## Introduction

Population growth in industrialized societies leads to greater wastewater and sewage sludge production, which threatens the environment (Shahbazi et al., 2017). Therefore, managing these materials has become a crucial environmental concern in recent decades (Rathod et al., 2009). In this regard, the cultivation of agricultural plants on soils amended with sewage sludge and fertilizers has significantly increased. Using sewage as an organic fertilizer is a globally practiced sustainable farm environmental management method (Asgari Lajayer et al., 2019). Dryland soils are characterized by poor organic matter content, weak structure, low fertility, and lack of water. However, the use of sewage sludge as a land amendment can have several positive impacts on soil properties. This includes adding organic matter to the soil, raising soil porosity, decreasing bulk

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density, improving soil drainage, increasing soil structural integrity, water retention, buffering ability, and enhancing the availability of macro- and micronutrients for crop. All these benefits contribute to improving crop productivity (Mohamed et al., 2018). On the other hand, some studies declared that sewage sludge can harm plant production, forage quality, and groundwater property. Unsuitable components are present in sewage sludge, including heavy metals such as Pb, Ni, Cr, As, Cd, Cu, Mo, and Zn. Additionally, organic compounds like pharmaceutical materials and pathogenic organisms must be closely monitored to ensure the safety of citizens' health (Cheraghi et al., 2015).

Several studies have analyzed the beneficial and harmful effects of sewage sludge on plant growth, on various agronomic plants (Kumar & Chopra, 2014; Mahmoudi et al., 2015). To minimize the potential transfer of pollutants from sewage sludge to the human food chain, it is recommended to use the sludge as fertilizer for non-food or forage crops. This way, the nutrients in the sludge can be recycled into crops that are not directly consumed by humans, reducing the environmental risks associated with sludge disposal (Sigua, Adjei & Jack Rechcigl, 2005).

Only some studies have investigated the impact of sewage sludge on the growth, quality, and heavy metal accumulation in forage plants. Gubišová et al. (2020) studied the effects of different concentrations of sewage sludge on the growth and biomass yield of giant reed (Arundo donax L.). Their findings showed that the number of shoots per plant was significantly higher when treated with 5 t ha<sup>-1</sup> of sewage sludge than the control. Lan (1988) conducted a study to investigate the effect of sewage sludge and water-soluble synthetic fertilizers on the forage quality and the absorption of metals by Bermuda grass. The result indicated that compared to commercial fertilizers, sludge increased the crude protein content of Bermuda grass forage. Moreover, copper density was twice as high in sludge-treated than in traditionally fertilized forage. The effects of periodic applications of sewage sludge indicated that all levels of applied sludges enhanced the forage production of Bahia grass more than the unfertilized control (Sigua, Adjei & Jack Rechcigl, 2005). Based on their results, sewage sludge can be used as a low-level nitrogen and phosphorus fertilizer and a source of calcium. In the investigation of the effects of sewage sludge and chemical fertilizer on natural pasture, the results indicated that forage dry weight, protein yield, and protein percentage increased with biosolid. The highest yield was obtained by applying 75 ton ha<sup>-1</sup> of biosolid and 150 kg N ha<sup>-1</sup> +75 kg  $P_2O_5$  ha<sup>-1</sup> of chemical fertilizer (Arvas et al., 2011).

Guar (*Cyamopsis tetragonoloba* (L.) Taub.) is a legume crop that grows mainly in dry and semi-arid regions, with a deep taproot structure that allows it to catch water from the soils, making it highly droughttolerant (Kapoor, 2014; Mahdipour-Afra et al., 2021). Guar seeds are primarily used to produce guar gum, widely used in the food, pharmaceutical, and beauty industries (Vaughan & Geissler, 1998). Guar seeds contain a considerable quantity of galactomannan gum, which makes guar a major industrial crop with more than 300 commercial usages (Mahdipour-Afra et al., 2021; Sharma et al., 2017). Guar plant residues are also used as a rich protein animal feed. The plant can also generate high-quality hay that can be used as a highly nutritious and tasty forage for the cattle (Kapoor, 2014).

The quality of guar is affected by various factors including the amount of nutrients in the soil. Chemical fertilizer, sewage sludge, municipal plant residues, and dredged substances can increase guar forage quantity and quality. However, the harmful consequences of these materials, especially sewage sludge, on guar forage quality should be considered. To our knowledge, no study has been conducted to monitor the effect of sewage sludge on the accumulation of heavy metal and forage quality of guar under field conditions. Against this background, we conducted a study based on the following hypotheses: (i) the utilization of sewage sludge has the potential to increase yield and quality of guar forage, and (ii) monitoring the treatments involving sewage sludge indicate that the concentration of heavy metals in both soil and above-ground plant parts remained below the toxic thresholds. The study aimed to assess (a) the effect of sewage sludge and chemical fertilizer on guar forage yield and its quality and (b) the environmental health risk of heavy metals in guar aboveground parts under different treatments.

#### Materials and methods

#### Field site description

The field experiment was conducted from March 2019 to October 2019 at the Yazd wastewater

treatment plant station  $(32^{\circ}00'N, 54^{\circ}19'E)$  on a sandy clay loam soil containing 52.52% sand, 30.34% clay, and 17.14% silt (Fig. 1).

The secondary sewage sludge was obtained from a sequential batch reactor (SBR) package at the Yazd wastewater treatment plant. The SBR process is a method of treating wastewater that performs all the functions of a conventional activated sludge plant. This includes biological removal of pollutants, separation of solids and liquids, and removal of treated effluent. The process uses a single variable volume basin that operates in an alternating mode, eliminating the need for final clarifiers and high-RAS pumping capacity (Chen et al., 2022). After treatment, the secondary sewage sludge is dried using air and then passed through a 2-mm sieve. Before sowing the seeds, an analysis of the sewage sludge was conducted. Table 1 shows the levels of nutrients and heavy metals applied through the sludge. Also, following the plant harvest, soil samples were collected from different treatments to evaluate the effects of sewage sludge on various soil properties.

#### Field experiment

The experimental design was randomized complete blocks with four replications to monitor the effects of four treatments on guar growth and accumulation of heavy metal in soil and plants. The treatments included sewage sludge (20 t ha<sup>-1</sup>), chemical fertilizer (NPK (20-20-20), 200 kg ha<sup>-1</sup>), combinations of sludge and chemical fertilizers (10 t ha<sup>-1</sup>sewage sludge +100 kg ha<sup>-1</sup> NPK), and nonfertilized control. The amounts of sewage sludge used was based on the Iranian National standard guideline (No. 10716) for solid waste and compost reuse in agriculture (Zazouli & Dehghan, 2014). The experimental field consisted of 4 by 3 m in size plots, with a 2-m weed-free margin through monthly roto-tillage during the growing cycle. In March 2019, the appropriate sewage sludge

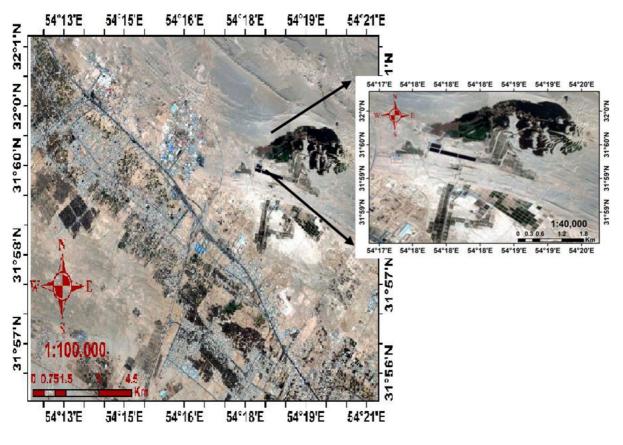


Fig. 1 Location of the study site at Yazd, Iran

	Total phos- pH EC (ds/m) Parameters phorus (%)	Sludge Sludge standard limit (USEPA503 2002)
	EC (ds/m)	6.53 6.94 
	μd	6.53 -
		3.16 -
	Total nitrogen Total potassium (%) (%)	0.51 -
	Total nitrogen (%)	4.02 -
the study	Organic mater (%)	47.06 -
sludge used ir	Pb (mg/kg)	3.21 300
f the sewage :	Cd (mg/kg)	0.41 39
Table 1Characteristics of the sewage sludge used in the study	Cr (mg/kg)	22.02 1200
Table 1 (	Ni (mg/kg)	22.7 420

and NPK treatments were roto-tilled and mixed into the topsoil. Guar seeded (10 kg  $ha^{-1}$ ) were sown by hand on April 25, 2019, with a row spacing of 50 cm. The field was irrigated twice a week using a flood irrigation system.

## Plant characteristics

In October 2019, the plants were harvested. A section of 1 m<sup>2</sup> from the center of each plot was harvested to calculate the amount of forage produced. Plant height was measured using a plastic ruler ( $\pm 0.1$  cm) from plant base to the tip (Sodaeizadeh & Mansouri, 2014). The length of the roots was measured using a ruler. The shoots and roots of the guar plants were separated, and their weight was measured with a digital balance ( $\pm 0.01$  g). The guar roots and shoots were then oven-dried at 70 °C until they reached a stable weight, after which the plant tissues were weighed individually and represented in gram per square meter for dry biomass determination.

Determination of heavy metal concentration

The soil heavy metals were extracted using the DTPA distillation procedure (Shahbazi et al., 2017). To prepare the solution, 96.1 g of DTPA was mixed with 3.13 ml triethanolamine solution, 47.1 g of calcium chloride, and 100 ml of distilled water. The pH of these solutions was adjusted to  $3.7 \pm 0.1$  using a pH meter. Finally, distilled water was added to the solution to make it up to 1 l in volume. A mixture of 25 ml of DTPA solution and 25 g of soil was shaken for 2 h. The resulting mixture was then filtered through grade 42 Whatman filter paper. A combination of nitric and hydrochloric acid distillate is used to extract heavy metals from sewage sludge (Ryan et al., 2001). A mixture of 1 g of sludge and 10 ml aqua regia was left at room temperature for 24 h. The samples were then heated to approximately 80 °C for 3 min to obtain a clear solution. After cooling, the samples were filtered entirely using the Whatman 42 filter paper. Deionized distilled water was added to the filtrate to reach a volume of 25 ml. The concentration of heavy metals in the soil and sludge was determined using an atomic absorption spectrometer (novAA® 350, Analytik-Jena AG, Jena, Germany). The above-ground parts of the plants were dried in an oven at 65 °C for 48 h to measure the amount heavy metals present. After drying, the samples were ground to pass through a 1-mm sieve. The dry ashing method (Westerman, 1990) was used to extract the heavy metals from the plants. One gram of each plant sample was weighed and placed in an electric furnace (Azar 1250 model, Azar Furnace Corp, the Netherlands) at 550 °C for 4 h. Then, 10 ml of 2NHCl was added to each sample, and mixture was heated until it was to one-thirds of its original volume. The mixture was allowed to cool before being filtered using Whatman filter paper (no. 42). The volume of the mixture increased to 50 ml by adding distilled water. An atomic absorption spectrophotometer determined the concentrations of Cd, Ni, Cr, Cu, Zn, Pb, and Mn. Each measurement was repeated four times to ensure accuracy.

## Forage quality determination

To evaluate the quality of forage various parameters such as crude protein (CP), crude fiber (CF), dry matter digestibility (DMD), neutral detergent fibers (NDF), acid detergent fiber (ADF), ash, and watersoluble carbohydrate (WSC) were measured. The plant samples were dried at 70 °C and ground into fine particles. A NIRS device (near infrared spectroscopy) model INFRAMATIC862 was used to measure 10 g of ground samples as described by Jafari et al. (2003) and Amini Hajiabadi et al. (2021).

## Statistical analysis

ANOVA statistical analysis determines significant differences between estimated variables across treatments. The treatment's mean was compared using Duncan's 5% significance level test. The SPSS Version 25 statistic software package was used for all statistical analysis. Data were presented as means  $\pm$  standard deviation (SD).

## Results

## Sewage sludge characteristics

Table 1 displays various properties of the sludge. The pH value of sewage sludge is slightly acidic. The salinity of sewage sludge is typically high at 6.94 dS/m, which is beyond the tolerance level of most seedlings. Leaching with water is a practical method to remove extremely soluble salts. According to Table 1, Pb, Cd, Cr, and Ni concentrations were found to be 3.21, 0.41, 22.02, and 22.7 mg/kg, respectively. Moreover, this study detected a high concentration of organic matter (47.06%) in the sewage sludge.

The monitoring of sludge application on soil nutrients and heavy metal contents

Table 2 displays various parameters of soil treated with different sludge and chemical fertilizer concentrations. The result indicates that when sludge is leached by flood irrigation, it reduces the sludge salinity without a significant increase in the salinity of treated soil (1.01-1.22 dS m<sup>-1</sup>) compared to the control (Table 2). The soils have a slightly alkaline pH (7.16-7.83) and a relatively high carbonate content (16.1-39.2%). Applying sludge to the soil resulted in a significant increase in soil organic matter. The consumption of 20 (t/ha) sludge resulted in a fourfold increase in soil organic matter compared to the nonfertilized control (Table 2). Additionally, the application of sludge to the soil resulted in a considerable increase in total nitrogen, potassium, and available phosphorus by 150, 13.7,, and 15.4%, respectively, compared to the control. Soil analysis also showed that the concentrations of heavy elements, including Pb, Co, Cr, Ni, Cu, and Zn, in all treatments, were lower than the World Health Organization (WHO) permissible limits for heavy metals in soil (Table 2).

The effects of sludge and fertilizer on some morphological characteristics of guar

Analysis of variance indicated that all studied traits were significantly affected (P < 0.01) by soil amendment treatments (Table 3).

Figure 2 presents the mean values of morphological characteristics of guar. The soil amendment treatments significantly impacted the height of guar plants. The application of nutrients from all sources resulted in significantly higher plant than the control. Additionally, applying sludge and sludge + fertilizer resulted in taller plants than the fertilizer alone. Root length of guar also affected by all soil amendment treatments and significantly increased compared to control. Plant dry weight was also affected by the studied treatments. The dry weight of the plants

Parameters	Control	Soil treated with 200 kg/ha NPK	Soil treated with 20 t/ha sludge	Soil treated with sludge +NPK	WHO threshold
EC (ds $m^{-1}$ )	0.86 ±0.09 a	1.01 <u>+</u> 0.01a	1.22 <u>+</u> 0.11a	1.15 <u>+</u> 0.12a	-
pH	7.16 <u>+</u> 1.1a	7.69 <u>±</u> 1.21a	7.83 <u>+</u> 1.18a	7.82±1.24a	-
CaCO <sub>3</sub> (%)	27.73±3.1c	16.1 <u>+</u> 2.2d	34.27 <u>+</u> 3.5b	39.9 <u>+</u> 3.91a	-
Organic matter (%)	0.3±0.03c	0.7±0.08b	1.2 <u>+</u> 0.11a	1.08 <u>+</u> 0.13a	-
Total nitrogen (%)	0.08±0.004c	0.15 <u>+</u> 0.01b	0.2±0.022a	0.18 <u>±</u> 0.03a	-
Available potassium (mg/kg soil)	160±8.25c	230±10.23a	182±11.3b	180 <u>+</u> 9.8b	-
Available phosphorus (mg/kg soil)	15.16 <u>±</u> 2.3b	17.85±2.11a	17.5 <u>+</u> 2.56a	18.19 <u>+</u> 2.68a	-
Pb (mg/kg soil)	1.1±0.12c	1.6±0.14b	2.1±0.21a	2 <u>+</u> 0.18a	85
Cd (mg/kg soil)	0.016±0.005b	0.017 <u>±</u> 0.008a	0.018±0.007a	0.017 <u>±</u> 0.005a	0.8
Cr (mg/kg soil)	3.45±0.38c	4.4 <u>±</u> 0.56b	5.4 <u>+</u> 0.85a	5.2 <u>+</u> 0.89a	100
Ni (mg/kg soil)	0.17 <u>+</u> 0.03a	0.17 <u>+</u> 0.021a	0.19 <u>+</u> 0.023a	0.18 <u>±</u> 0.03a	35
Cu (mg/kg soil)	0.13±0.022c	$0.2 \pm 0.08 b$	0.37 <u>±</u> 0.06a	0.22±0.069b	36
Zn (mg/kg soil)	0.31±0.065c	0.47 <u>±</u> 0.081b	0.64 <u>±</u> 0.097a	0.6 <u>±</u> 0.098a	50

Table 2 Effects of soil amendment treatments on some physico-chemical properties of soil

Table 3 Analysis of variance the effects of soil amendment treatments on some morphological characteristics of guar

Mean square										
Sources of variation	Degrees of freedom	Plant height	Root length	Shoot dry weight	Root dry weight					
Block	3	24.35 <sup>ns</sup>	3.4 <sup>ns</sup>	1221.7 <sup>ns</sup>	217.8 <sup>ns</sup>					
Soil amendment treatments	3	840.33**	38**	264085**	7542**					
Error	9	22.2	2.2	4650.7	497.7					

\*\*Significant at the level of 0.01. ns = non-significant

ranged from 629 g m<sup>-1</sup> in the control treatment to 1050 g m<sup>-1</sup> in the sludge + fertilizer plots. The root dry weight followed a similar trend to root length and nutrients from different sources significantly improved this parameter compared to the control (Fig. 2).

Monitoring the effects of sludge and fertilizer on some heavy metal concentrations in above-ground parts of guar

Results from the analysis of variance indicated that all studied heavy metal concentrations in above-ground parts of guar were significantly affected (P < 0.01) by the soil amendment treatments (Table 4).

Application of all soil amendment treatments (except at fertilizer for Cd) increased plants' heavy metals compared to the control soils (Table 5). Our results indicated that maximum amounts of Cd  $(1.5 \text{ mg kg}^{-1})$  and Cr  $(4.9 \text{ mg kg}^{-1})$  were observed in sludge + fertilizer treatment. No statistically significant variations were identified in the Cd and Cr concentrations between plants grown in sewage and sludge + fertilizer. Although sludge application increased Cd and Cr concentrations, their levels remained within the normal range and below the toxic limits (Table 6) according to Shahbazi et al. (2017). Table 5 shows that the sludge + fertilizer had the highest concentrations of Cu, Mn, Pb, and Zn which were 131, 62, 300, and 43% higher than the control. However, similar to Cd and Cr, these concentrations were within the normal range (Table 6). All soil treatments significantly increased Ni concentration in above-ground guar parts (Table 5); but it remained below toxic levels (Table 6).

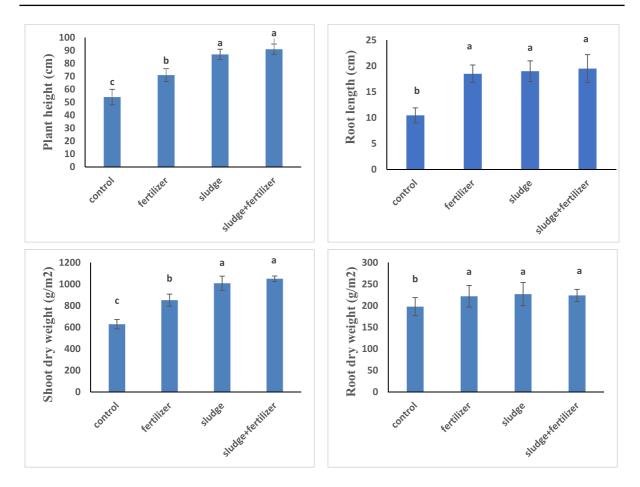


Fig. 2 Effect of soil amendment treatments on some morphological parameters of guar (mean  $\pm$ SD). Means with at least one common letter are not significantly different at the level of 0.05 of Duncan test

Table 4	Analysis of varian	ce the effects of soi	il amendment	treatments on	guar heav	y metal concentration

Mean square									
Sources of variation	Df	Cd	Cr	Cu	Mn	Ni	Pb	Zn	
Block	3	0.035 <sup>ns</sup>	0.12 <sup>ns</sup>	3.2 <sup>ns</sup>	12.65 <sup>ns</sup>	0.076 <sup>ns</sup>	0.035 <sup>ns</sup>	7.24 <sup>ns</sup>	
Soil amendment treatments	3	0.355**	$8.56^{**}$	31.08**	432.97**	1.138**	3.88**	653.12**	
Error	9	0.02	0.092	1.23	14.98	0.066	0.046	5.68	

\*\*Significant at the level of 0.01. ns = non-significant

Treatments	Heavy metals										
	Cd	Cr	Cu	Mn	Ni	Pb	Zn				
Control	0.7±0.08b	1.6±0.38c	5.8±0.83c	45±2.5d	2±0.35b	1±0.56c	23±1.48d				
Fertilizer	$0.9 \pm 0.07 b$	3.3±0.3b	8.9±0.48b	53±3.2c	3±0.3a	3±0.32b	32±2.17c				
Sludge	1.3 <u>±</u> 0.09a	5.2 <u>±</u> 0.2a	10.9±1.5b	60±3.5b	3±0.22a	3±0.11b	48±3b				
Sludge + fertilizer	$1.5\pm0.08a$	4.9±0.27a	13.4±1.3a	73±5.7a	3±0.03a	4±0.26a	56±2.6a				

Means in the same column followed by different letters are significantly different at P < 0.05 Duncan test

Metal	Normal range	Toxicity range
Cu	5-20	20-100
Zn	1-100	1–10
Mn	5-100	>100
Cd	0.1-2.4	5-30
Pb	0.2–20	30-300
Cr	0.03-0.14	5-30
Ni	0.02–5	10-100

**Table 6** Normal and toxic values from heavy metals observed in plants (mg/kg) (Shahbazi et al., 2017)

Results from the analysis of variance indicated that soil amendment treatments significantly affected some forage quality parameters, such as CP, DMD, and WSC, while CF, ADf, NDF and Ash were not significantly impacted (Table 7).

Based on our findings, the sludge + fertilizer treatment resulted in the highest crude protein level of 14.1% in guar, which was significantly higher than the control, fertilizer, and sludge treatments by 8.8, 4, and 2.4 times, respectively (Table 8). We also observed that the sludge + fertilizer amendment significantly increased the digestible dry matter (DMD) compared to other treatments. However, there was no significant difference between the other three soil amendment treatments (Table 8). The study found that soil amendment treatments did not have a significant effect on acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Table 8). However, the sludge and sludge + fertilizer amendments resulted lower levels of ADF and NDF. The soil amendment treatment also significantly affected the guar water-soluble carbohydrates (WSC) content. WSC ranged from 16% in the sludge + fertilizer amendment to 14.1% in the control (Table 8).

### Principal component analysis

According to the principal component analysis (Fig. 3), the measured parameters were classified into two separate clusters based on Pearson correlation. Cluster 1 includes WSC, DMD, CP, Ni, Pb, Cu, Cr, Mn, Zn, Cd, plant height, root length, and shoot dry weight; all positively correlated. Cluster 2 includes NDF, ADF, and root dry weight. The results showed a positive correlation between sludge and sludge + fer-tilizer with cluster 1 and a negative correlation with cluster 2.

Table 7 Analysis of variance the effects of soil amendment treatments on forage quality parameters of guar

Mean square									
Sources of variation	Df	Crude fiber (CF)	Crude protein (CP)	Acid detergent fiber (ADF)	Neutral detergent fiber (NDF)	Digestible dry matter (DMD)	Ash	Water-soluble carbohydrates (WSC)	
Block	3	2.135 <sup>ns</sup>	0.22 <sup>ns</sup>	1.3 <sup>ns</sup>	3.65 <sup>ns</sup>	0.176 <sup>ns</sup>	0.06 ns	0.21 <sup>ns</sup>	
Soil amendment treatments	3	2.52 <sup>ns</sup>	$1.61^{*}$	2.17 ns	4.4 <sup>ns</sup>	$1.41^{*}$	0.21 ns	$2.42^{**}$	
Error	9	2.34	0.354	0.69	2.23	0.189	0.168	0.282	

\* and \*\* significant at the level of 0.05 and 0.01, respectively. ns = non-significant

Table 8 Effect of soil amendment treatments on forage quality parameters of guar

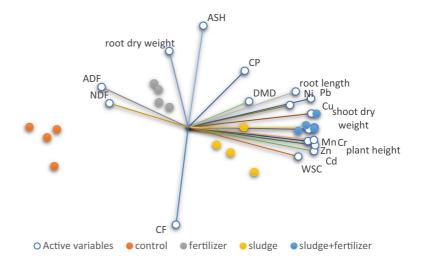
Treatments	Crude fiber (%CF)	Crude protein (%CP)	Acid detergent fiber (%ADF)	Neutral detergent fiber (%NDF)	Digestible dry matter (%DMD)	ASH(%)	Water-soluble carbohydrates (%WSC)
Control	31.1 <u>+</u> 2.13a	12.9 <u>+</u> 0.87b	30.9±1.42a	34.6±1.12a	62.2 <u>+</u> 3.22b	10.3±1.28a	14.1±1.08b
Fertilizer	29.3 <u>+</u> 1.77a	13.5 <u>+</u> 2.39b	30.9 <u>+</u> 1.06a	34.2 <u>+</u> 2.39a	62.4b±2.49	10.9 <u>±</u> 1.18a	14.2 <u>+</u> 0.97b
Sludge	31.8 <u>+</u> 2.68a	13.7±1.86b	30.3 <u>+</u> 0.58a	335±1.01a	61.7 <u>±</u> 2.11b	10.3±1.33a	15.6 <u>+</u> 1.58a
Sludge+fertilizer	29.9 <u>+</u> 2a	14.1±1.22a	29.4 <u>+</u> 2.48a	33.4 <u>+</u> 0.79a	63.4 <u>+</u> 3.39a	10.6 <u>±</u> 0.91a	16 <u>±</u> 0.94a

Means in the same column followed by different letters are significantly different at P < 0.05 Duncan test

#### Discussion

The use of sewage sludge as an organic fertilizer is a commonly used environmental management technique in agricultural lands around the world. This study aims to monitor concentrations of heavy elements in soil and guar plants. These heavy elements can harm animal and human health if they enter the food chain through the soil, plant system. According to our results, salinity level in sludge is relatively high. Water leaching is a practical method to remove extremely soluble salts from the sewage sludge, as suggested by Liu et al. (2014). In this study, flood irrigation was carried out before sowing to reduce the salinity of the sludge. Therefore, the application of sewage sludge did not significantly increase soil salinity. Noguera et al. (1997) indicated that irrigation effectively leaches extra soluble salts from organic waste used as a substrate. After comparing the levels of heavy elements in the sewage sludge used and the standards set by the US Environmental Protection Agency (USEPA 503) (2002), it was discovered that the concentration of the heavy metals in the sludge is lower than the standard level. The metal properties and physicochemical characteristics of the sludge under study confirm that it can be used as a replacement for fertilizer. In this study, sludge applications significantly increased soil organic matter, making it a viable alternative to organic fertilizers. Most of Iran's arable farmlands are typically rich in lime but have low organic matter content and biological activity in the topsoil. Therefore, using sewage sludge as an organic amendment and nutrient source in these soils, with a relatively low risk of pollution, can improve their physico-chemical and biological traits (Bozkurt et al., 2010). Organic matter plays a crucial role in the uptake of metals by soil, which means that increased levels of organic matter can decrease the mobility of soil metals (Hamdi et al., 2019). Several studies indicated that sewage sludge can increase soil organic matter and nutrient availability (Farsang et al., 2020; Zuo et al., 2019). Our results indicate that soil treated with sludge is not acidic, which means that absorption of metals by plants is not restricted in this study. These findings are consistent with those of Romanos et al. (2021), who found that pH values were not a limiting factor in their study. Our research also confirms that sludge utilization increases soil macronutrients. Many studies have revealed that using sewage sludge as a crop yield enhancer provides plants with valuable nutrients (Eid et al., 2020). Sludgeamended soil, rich in macronutrients like N, P, and K, is produced by microorganisms that alter the mineral structure of composted sludge, making these nutrients available to plants (Diacono & Montemurro, 2011). Soil analysis also showed that the concentrations of heavy elements were lower than the World Health Organization (WHO) permissible limits. Two factors could be responsible for the low concentration of heavy metals in soil after a harvest. One is the leaching of heavy metals, as noted by Conde-Suárez et al. (2004), and the other is the absorption of these metals by plants, as reported by Eid et al. (2020). In this study, using sludge and commercial

Fig. 3 PCA of measured traits of guar in response to fertilizer, sludge and sludge+fertilizer



fertilizer significantly improved the root length, plant height, and shoot and root dry weight compared to the control. This enhancement in the morphological characteristics of guar plants can be attributed to the improved soil fertility resulting from sludge utilization. Our findings suggest that while chemical fertilizer positively impacted the height and dry weight of guar plants, it was not as effective as sewage sludge. This indicates that plant nutrients, particularly nitrogen from sewage sludge, significantly influence crop growth more than chemical fertilizers (Kchaou et al., 2018). Azam and Lodhi (2001) reported that applying sewage sludge has advantages due to releasing nitrogen from the decomposition of organic materials with high nitrogen content and low carbon-nitrogen ratio. Asgari Lajayer et al. (2019) suggested that the improved growth parameters of basil under the sludge treatments may be due to the higher amounts of macro- and micronutrients, which create more favorable conditions for plant growth. Mohamed et al. (2018) indicated that the enhanced above-ground yield of Moroccan sunflower could be related to the source of N and P for plant in sludge. Other investigators have also noted sludge's positive effects on plants' different growth parameters (Fresquez, Francis, & Dennis, 1990; Prinzenberg et al., 2010).

This study showed that application of all soilamended treatments led to an increase in heavy metals concentration in plants compared to the control. However, despite the increased heavy metal concentration due to the sludge application, the levels remained within the normal range and did not exceed the toxic concentration. The concentration of cadmium, the most harmful metal component, depends on its density in sewage sludges, soil pH, plant species, and weather conditions (Singh & Agrawal, 2008). The uptake of cadmium by plants is a serious health concern in the food chain, from soil to plant to human. Our research has shown that Cd and Cr concentration in soil and plants are within normal limits. This is significant because concerns over the concentration of heavy metals, especially Cd levels, often limit the use of sewage sludge in agriculture. Although the maximum concentrations of Cu, Mn, Pb, and Zn were recorded in the sludge + fertilizer, the concentration of these four elements were still within the normal range. Various factors affect the concentration of Cu, Mn, and Zn in plants, including the amount of metal that can be absorbed, the absorption rate, the transportation of these metals from root to stem, and the plant's growth rate (Najafi & Towfighi, 2008). The sludge + chemical fertilizer treatment increases the concentration of the elements mentioned above because the chemical fertilizer stimulates the microbial decomposition of organic materials in the sludge. This process causes some metals to be released into the soil, which, in turn, absorbed in greater amounts by plant roots. Mahal et al. (2019) reported that using chemical fertilizers significantly increased the decomposition and mineralization of soil organic matter. Different studies have examined the heavy metal concentration in plants grown in soil treated with sewage sludge. The aggregation pattern varies depending on soil properties, plant physiology, and the chelating effects of other metals. Only the high-concentration and frequently used sludge, when added to soil, can enhance heavy metal concentrations in soil and plant tissue (Koupaie & Eskicioglu, 2015; Yagmur et al., 2017). Dede and Ozdemir (2016) found that Cd concentration in the stem of Conyza canadensis increased significantly with sludge treatments. Hernandez et al. (1991) concluded that plants grown in sludge-treated soil contained significantly higher quantities of Fe, Cu, and Zn than those grown in control soil. Limam et al. (2018) reported that using sewage sludge as a soil amendment can increase the amount of soluble ligands in the soil, subsequently increasing the mobility of metals.

Crude protein percentage is a crucial and effective indicator of forage quality. Our study showed that the highest amount of crude protein in guar was observed in sludge + fertilizer treatment. This suggests a more consistent supply of nitrogen from the organic source throughout the season. Since the amount of forage protein is highly dependent on the amount of nitrogen in the soil, it is expected that significant amount of nitrogen from chemical fertilizer will be available at the beginning of the season. In contrast, only a small amount would be available at the end of the season which could affect the forage quality (Lan, 1988). Our results suggest that using sludge + fertilizer led to a higher protein content in guar forage compared to sludge treatment alone. This could be attributed to the microbial decomposition of organic materials in the sludge by chemical fertilizer, which releases nutrients, particularly nitrogen, into the soil. As a result, the plant roots absorb more amounts of these nutrients. Khiabani and Celen (2020) also found similar results

where the crude protein contents of vetch + barley mixture was higher in sludge and mineral fertilizer applications than in control. Increasing crude protein by utilization of sludge and organic fertilizer has been reported by other investigators (Hakl et al., 2021; Lan, 1988). In this study, digestible dry matter (DMD) of guar was significantly increased when soil was treated with sludge + fertilizer. Forage quality is heavily dependent on the percentage of DMD. Livestock prefer forage with higher digestibility, which provides more energy and nutrients to the animals (Karimian & Ebrahimi, 2017). Therefore, digestibility plays a crucial role in determining the overall forage quality. Similar to our results, Truba et al. (2020) reported that the highest digestibility of cocksfoot was achieved when both mineral fertilizers and compost (organic fertilizer) were used together. The forage DMD is inversely correlated with the amount of cell walls (Jung & Lamb, 2003) and lignin present. Therefore, it can be inferred that positive effect of sludge + fertilizer application on DMD in guar is due to the lower severe lignification of cell walls. Our results also showed that acid detergent fiber (ADF) and neutral detergent fiber (NDF) levels were relatively lower in the soil treated with sludge and sludge + fertilizer amendments. ADF consists of cellulose, lignin and hemicellulose, which can negatively impact the forage quality. When ADF levels are high, it results in low forage digestibility and energy contents (Ali et al., 2019; Gallegos-Ponce et al., 2012; Moreno-Reséndez et al., 2017). ADF and NDF fractions can limit animal dry matter intake, digestibility, and energy. Previously Ali and Tahir (2021) found that organic fertilizer had slightly lower ADF and NDF contents than inorganic fertilizer. The decrease in NDF and ADF rates could be attributed to the high nitrogen and protein content rate of aboveground parts of guar in the soil treated with sludge and sludge + fertilizer amendments compared to control (Khiabani & Celen, 2020). The highest and lowest water-soluble carbohydrates (WSC) levels were observed in sludge + fertilizer amendment and control, respectively. Proteins, carbohydrates, and lipids are the three primary nutrients affecting forage crops' nutritional value. These nutrients provide the necessary energy for animals and determine the digestibility of crops (Van Soest, 2018). Carbohydrates, in particular, are highly significant from a nutritional perspective (Kung et al., 2010).

The WSC percentage in forage crops can be affected by different factors, such as the type of fertilizer used. Generally, applying fertilizer to the soil can impact the quality of forage. Li et al. (2016) found that CP and WSC amounts in forage wheat increased significantly as nitrogen utilization increased. The quantity of WSC in plants depends on the amount of nitrogen in the soil. In this study, the use of sludge + fertilizer increased the amount of soil nitrogen absorbed by the guar plant, leading to an increase in the amount of WSC in its forage. This study suggests that using sewage sludge could be a cost-effective method to increase the yield and quality of guar without increasing concentration of heavy element in the soil and plants to toxic levels. However, it is crucial to remember that the characteristics of sewage sludge can vary significantly based on factors such as its origin, environmental conditions, and the treatment process used. As a result, it is essential to monitor the concentration of heavy metals in soil treated with sewage sludge periodically, especially in environmental situations that differ from those in the current study, to ensure that the levels of heavy metals do not exceed safe limits.

#### Conclusion

Applying sewage sludge to soil is a valuable way to recycle nutrient and reduce the need for commercial fertilizers in agricultural lands. Our experimental findings support the hypotheses that (1) application of sewage sludge increased yield and quality of guar forage by improving soil fertility and (2) monitoring sewage sludge application indicated that using sludge caused heavy metals to accumulate in the soil and plants. While these concentrations were within in the normal range and not toxic. Overall, the results of the present study suggest that sewage sludge can be a sustainable alternative to mineral fertilizers for guar cultivation. The use of sewage sludge as fertilizers has the potential to reduce waste, recycle nutrients and organic matter back into the soil, and promote sustainable development by modifying the utilization of natural resources in the research area under study. However, further research is needed to evaluate the long-term effects of sludge accumulation on soil and plant quality.

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**Data availability** The data that support the findings of this study are available from the corresponding author up on reasonable request.

#### Declarations

**Ethics approval** All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors

Consent to participate Not applicable.

Consent for publication Not applicable.

**Competing interests** The authors declare no competing interests.

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