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Assessment of the Health Risk Induced by Accumulated Heavy Metals from Anaerobic Digestion of Biological Sludge of the Lettuce

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Abstract

Heavy metals are a group of pollutants in biological sludge. Many agencies regulated guidelines for heavy metal concentrations for various applications of sludge such as agricultural application. In this study, we tried to determine heavy metal fate after anaerobic digestion. Additionally, we determined the bioaccumulation rate of heavy metals in lettuce cultivated on a sludge-applied land. Heavy metal (As, Pb, Hg, Cd) contents of solid and liquid parts of raw and anaerobically digested sludge were separately measured by ICP-OES. For this purpose, the samples were digested using nitric acid, hydrochloric acid, and boric acid. Then, the raw and anaerobically digested sludge were used for cultivation of lettuce in separate farms. The heavy metal concentrations in the harvested lettuce were measured by the same procedure. The results showed that the main part of heavy metals in the raw sludge was in the liquid part (67%), while, the main part of heavy metals in the anaerobically digested samples was in the solid part of the sludge. Because of washout of dissolved heavy metals in the liquid part of the sludge, the lettuce cultivated by anaerobically digested sludge had higher content of the heavy metals in comparison to that of the lettuce cultivated by the raw sludge. This study showed that application of anaerobically digested sludge can increase the bioaccumulation rate of heavy metals in the crops and induce more human health risk.

Keywords Heavy metals · Bioaccumulation · Health risk assessment · Anaerobic digestion

Abbreviations

EU	European Union
HQ	Hazard quintet
HI	Hazard index
ADI	Average daily intake
ED	Exposure duration
RfD	References dose
Bw	Body weight
WAS	Waste-activated sludge
As	Arsenic
Pb	Lead
Hg	Mercury
Cd	Cadmium
ICP-OES	Inductively coupled plasma-optical emission spectrometry
RPM	Revolutions per minute
μm	Micrometer
LOD	Limit of detection
LOQ	Limit of quantification

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Introduction

One of the main by-products of wastewater treatment plants is biological sludge [1]. The biological sludge contains about 90% water and is extremely biodegradable [2]. Various aerobic and anaerobic digestion processes can be employed for sludge digestion and stabilization [3]. The application of sludge anaerobic digestion has increased around the world,

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due to this process advantages. Some of these advantages include significant reduction of the sludge volumes resulting in the facilitation of the final disposal, producing biogases, energy recovery, and removal of the inappropriate matters such as pathogens or some persistence compounds. Mesophilic (35–40 °C) or thermophilic (55–60 °C) digestion with high and/or low solid contents is a various type of anaerobic digestion operation [4–6]. Usually, anaerobic digesters are operating in mesophilic mode, because this is more stable than the thermophilic one and also needs lower energy [6].

The amount of digested and treated biological sludge has increased around the world considerably [7, 8]. Therefore, to appropriately manage the massive volume of sludge, there is a need to find a sustainable transformation method to transform raw sludge to a stable material. After that, the stabilized sludge is applicable in required fields. One of the main applications is land application in the farms. Besides the biodegradable component, the important point in land application of raw or digested biological sludge is the existence of some treating components, like pathogens, heavy metals, and a wide range of organic pollutants.

The significance of heavy metals is their potential to accumulate in crops and thus enter the human food chain and threatening public health [9]. Thus, the European Union (EU) Directive 86/278/EEC, CEC [10], has put in place several limitations for heavy metal concentration to safe application of digested wastewater sludge in land. The hazardous elements having restriction include cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg). Previously, the significant issue about sludge quality was based on total heavy metal concentration. However, now, there are a lot of data and evidences indicating bioavailability and toxicity of these elements is individual [10]. Therefore, today, it is accepted that individual concentration of each heavy metal in the digested sludge is needed for the evaluation of possible environmental and human impact of its application in the farm [11].

The physicochemical processes and biological activities of microorganisms in activated sludge treatment process can absorb and accumulate heavy metals from the wastewater [12]. Therefore, by frequent application of raw or digested sludge in farms, the concentration of heavy metals can be increase in cultivable layers of the soil followed by increasing in the crops.

Anaerobic digestion processes can reduce organic contents of digested biological sludge but its behavior about transformation of heavy metals between liquid and solid parts of sludge could differ [13]. Therefore, the fate of heavy metals in anaerobic digestion of sludge is important for determination of heavy metal load in land by application of anaerobic digested sludge. In this study, we tried to determine effects of anaerobic digestion of activated sludge on heavy metal content of digested sludge and its susceptibility for agricultural usage and evaluation of its ecotoxicity.

Next, we try to determine differences between application of raw and anaerobic digested sludge on heavy metal bioaccumulation in the soil and in leafy vegetables. Finally, we try to investigate on differences between these two methods in produced health risk.

Material and Methods

Sample Preparation and Digestion

Waste-activated sludge (WAS) samples were obtained from a wastewater treatment plant in south of Tehran, Iran. WAS samples were collected from influent (raw sludge) and effluent (anaerobic digested) of the six anaerobic digester units every month over a 1-year period from March 2016 to March 2017. The samples were in volume of 5 l. The number of captured samples was 144 and they were stored at 4 °C before experiments.

The dewatering was used for all raw- and anaerobic-digested samples to extract bioleaching and liquids of samples. During the first step, the samples were divided into five equal parts (1 l), then 200 ml of each part was dewatered by centrifugation at 5000 rpm. Separated liquid phase and remained solids were collected in different containers. The remained solids were dried in an oven (80 °C) during 24 h. Then, the samples were weighted.

The dried solids from each sample were weighted. A high-performance microwave digestion system (ETHOS UP, milestone, USA) was used for digestion of samples. 0.2 g of dried solids was weighed and added to the digester vessels. Two kinds of acids including 65% nitric acid (m/m) and hydrochloric acid (37% m/m) were added to the vessels and placed in the digester for programmed period of time with controlled temperature and pressure. After that, the vessels were cooled for 30 min followed by adding 2 ml boric acid (H_3BO_3). The vessels were closed once more and irradiated 10 min. Next, the vessels were cooled and centrifuged. Remained solution was diluted by distillated water to volume of 50 ml and was introduced to the ICP-OES. The analysis of each sample was repeated three times. Before the analysis of samples, the ICP-OES was calibrated by standard stock solutions, introduced by its company (SPECTRO ARCOS - Germany) and R^2, LOD, and LOQ were achieved for every metal (Table 1).

Separated liquids from each sample were filtrate by a 0.45-μm fiberglass filter to remove trace that remained suspended colloids. Then, the heavy metal concentration in the filtrate was measured by ICP-OES (SPECTRO ARCOS, Germany).

Bioaccumulation

To determine the accumulation rate of heavy metals by application of sludge in farms, two controlled experimental lands with dimension of 3 × 3 m were chosen. To compare the

Table 1 ICP-OES analytical features for measurement of four heavy metals

Metal	R^2	LOD (μg l^{-1})	LOQ (μg l^{-1})
As	0.99984	1.187	3.9171
Pb	0.99964	2.166	7.1478
Hg	0.99968	0.351	1.1583
Cd	0.99943	0.04875	0.160875

transfer rate of heavy metals from sludge to soil and from the soil to vegetables, lettuce was chosen as a leafy vegetable. Eighty-one bushes of lettuce were planted in each farm. After 4 weeks, 20 l of anaerobic digested and dewatered activated sludge with 8% moisture added to the land 1. In other land, 20 l of raw sludge by 96% moisture was added. The added raw and digested sludge were taken from the influent and effluent of digesters in October.

After 10 weeks, the bushes of lettuce were harvested. In farm 1, the mean of bushes weight was 1200 g. All of the 81 bushes were gridded and dried in the oven 80 °C for 48 h. After the drying period, the total weight of dried sample was 4860 g. This main sample was divided into five samples by weight of 950 g. The digestion of samples was difficult because of the large volume in them. Thus, the samples were introduced to a furnace to make ash in 700 °C for 4 h. During combustion, the exhaust of furnace was connected to an impinger including 5% solution nitric acid (10 ml) to adsorption of vaporized arsenic and mercury. Next, the remained ashes was weighted and it was established that10 g of the ashes was digested according to the procedure explained above. The impinger solution was concentrated by evaporation of solution with a hot plate (30 °C) to 1 ml. Both the digested samples and the concentrated solutions were introduced to ICP-OES.

This procedure was done for the lettuce harvested from land 2. The mean of bushes weight harvested from land 2 was 1170 g. After drying, the total weight of the dried sample was 3670 g. The four samples with weight of 910 g were prepared from the main sample. Ashing and digestion steps were done as explained above.

To determine the accumulation of heavy metals in the soil, after harvesting of bushes, 10 samples including 2-g soils from top layer and 10-cm depth were collected. Digestion procedure was done as above and the samples were introduced to the ICP-OES.

To conduct a health risk assessment following the ingestion of these lettuces, first, the average daily intake of each metal was calculated by Eq. 1. Then, the hazard quotient was calculated by Eq. 2:

$$\mathrm{ADI} = \frac{C \times I \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \quad (1)$$

where ADI is the average daily intake by digestion of vegetables (mg kg^{-1} day^{-1}), C is the metal concentration in the vegetables (mg kg^{-1}), I is the ingestion rate (kg day^{-1}), EF is the exposure frequency, ED is the exposure duration (year), BW is the body weight (kg—supposed 70 kg), and AT is the exposure time period.

$$\mathrm{HQ} = \frac{\mathrm{ADI}}{\mathrm{RfD}} \quad (2)$$

where HQ is the hazard quintet and RfD is the reference dose for ingestion (mg kg^{-1} day^{-1}). Finally, the hazard index (HI) was calculated by Eq. 3.

$$\mathrm{HI} = \sum \mathrm{HQi} \quad (3)$$

Used oral reference doses for calculation of HQ are determined by USEPA and are 1×10^{-3} mg kg^{-1} day^{-1} for cadmium, 3×10^{-4} mg kg^{-1} day^{-1} for arsenic, and 0.35×10^{-3} mg kg^{-1} day^{-1} for lead [14, 15].

Statistical Analysis

Two descriptive plots including heatmap plot of metal concentrations (Fig. 1) and hazard quotient of metals (Fig. 2) have been drawn. Statistical tests such as one-way ANOVA test and paired t test have been done at a level of 0.05. Therefore, we assume p values less than 0.05 statistically significant. All the statistical analysis was carried out in R software (3.5.0).

Results and Discussion

The means of total concentration of heavy metals in raw sludge (solids and liquids) during the experiment period is shown in Fig. 1.

Also, Table 2 shows proportion of heavy metal concentration in the solid part of the sludge to total concentration of heavy metals in raw and anaerobically digested sludge. As

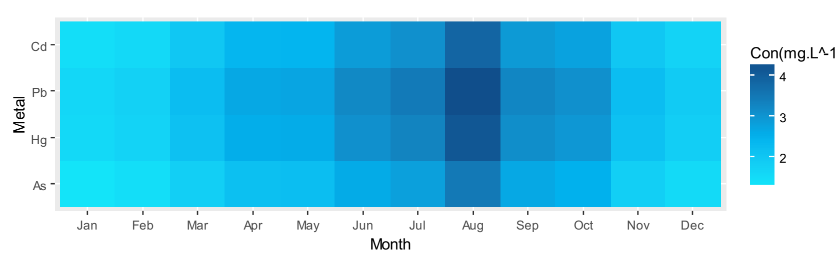

Fig. 1 The means of heavy metals concentration in raw-activated sludge (mg l^{-1})

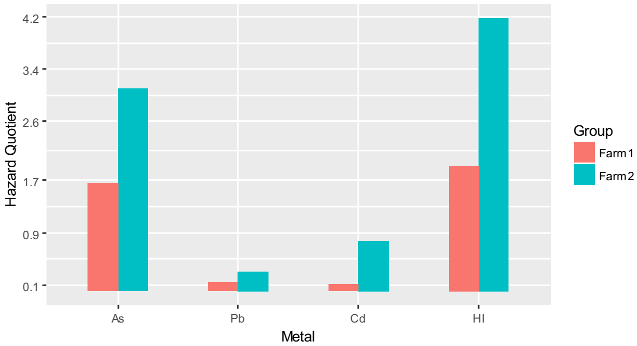

Fig. 2 Attributed risk and hazard index for all heavy metals in both groups of harvested lettuce

shown in Table 2, the main part of heavy metals is in the liquid part of the sludge. Thus, the concentration of raw sludge by dewatering methods, such as centrifuge, can decrease the heavy metal content of sludge. Moreover, concentration of sludge can facilitate sludge transfer and management. A notable problem in this procedure is the maintenance of produced leachate containing heavy metals [16].

The one-way ANOVA analysis was applied to examine the heavy metal concentration variation in biological produced (raw) sludge in different seasons. Achieved *P* values show significant differences in heavy metal concentration of raw sludge in different seasons. In order to determine the source of difference, we compared the heavy metal concentration of raw sludge in each pair of seasons. In this case, the Tukey HSD test has been implemented and the results of the test are shown in Table 3.

As shown in Fig. 1, the maximum value of the heavy metal concentration in activated sludge occurred in August. García-Delgado et al. [17] informed that the max level of heavy metals occurs in the summer months [17]. In contrast, Kasina et al. [18] mentioned that there are two peaks for heavy metal concentration in winter and summer and the main peak is winter peak [18]. In the current study, only one peak was observed in the late of summer. The clear point is that heavy metal concentration in the wastewater and finally in the produced activated sludge has a periodic pattern. As mentioned in Table 3, the concentration of heavy metals in different seasons has significant differences. Thus, some factors such as the type of wastewater, collection system, (separate or combined) concentration in runoff and soils, and industrial emissions can affect the concentration [19]. Therefore, because of various sources of heavy metals in the biological sludge, measurement and attention to the fate of these elements in the sludge processes in different seasons of year are necessary for the safe application of digested sludge.

Table 4 shows the concentration of heavy metals in anaerobic-digested sludge with concentration in the inlet raw sludge. The concentration was reported in the solid and liquid parts of the samples separately.

The paired *t* test for examination of differences between heavy metal concentrations in the solid part of the sludge shows that heavy metal concentration in the solid part of the sludge is significantly increased after anaerobic digestion in all season (P value < 0.001). As Inyang et al. had mentioned, beside bioaccumulation of heavy metals in the bacteria's body, it can be adsorbed by organic absorbent in the sludge [20]. Thus, several studies reported that dewatering of activated sludge after anaerobic digestion can reduce heavy metal content of the remained liquids. Comparison of remained heavy metals in the solid part of the digested sludge by some guidelines such as European Union (EU) Directive 86/278/EEC shows that the digested and dewatered sludge can be used for agricultural purpose safety. It must be mentioned that EU Directive 86/278/EEC has no limitation value for arsenic that must be taken into account [21].

Bioaccumulation Assessment

Table 5 summarized the load rate of heavy metals to the farm 1 and farm 2, their concentration in the top and 15-cm depth layer of soil and harvested lettuce, and also the bioaccumulation rate of heavy metals.

The ANOVA statistical analysis on the concentration of heavy metals in top and 15-cm depth layer of the soil after harvesting shows significant differences between heavy metal concentrations in farms 1 and 2 (P value < 0.05). Also, this statistical analysis was performed on heavy metal concentration in harvested lettuce from both farms and shows significant differences in concentration between them and also in bioaccumulation rate. In application of raw sludge (farm 1), the bioaccumulation rate is regarding to As > Pb > Hg > Cd

Table 2 Mean concentration of heavy metals in solid and liquid parts of raw- and anaerobic-digested sludge (± SD)

Metals concentration (mg/kg)	Raw sludge				Digested sludge			
	As	Pb	Hg	Cd	As	Pb	Hg	Cd
Solid part	0.37	0.6	0.4	0.6	1.7	2	1.9	1.77
Liquid part	2.13	2.4	2.5	2.1	0.8	1	1	0.93
Total	2.5 (± 0.21)	3 (± 0.16)	2.9 (± 0.16)	2.7 (± 0.17)	2.5 (± 0.21)	3 (± 0.16)	2.9 (± 0.16)	2.7 (± 0.17)

Table 3 Significance of differences in heavy metal concentration in the sludge in different seasons

Season	As	Hg	Pb	Cd
Spring-autumn	< 0.05	< 0.05	< 0.05	< 0.05
Summer-autumn	< 0.001	< 0.001	< 0.001	< 0.001
Winter-autumn	< 0.001	< 0.001	< 0.001	< 0.001
Summer-spring	< 0.001	< 0.001	< 0.001	< 0.001
Winter-spring	< 0.001	< 0.001	< 0.001	< 0.001
Winter-summer	< 0.001	< 0.001	< 0.001	< 0.001

and in application of anaerobic digested sludge (farm 2), the direction of rates was Hg > As>Pb > Cd, although, in both case, differences in rates are not significant. But as mentioned above, differences between both farms are strongly significant. Regarding the same condition of both farms and same total load rate of heavy metals in both farms, the main factor that enhanced accumulation rate in farm 2 could be biosolid susceptibility to keep heavy metals in complex form and introducing them to the soil slowly. As Liu et al. [22] mentioned, substitution of heavy metals by vital metals such as Zn and Cu in the enzyme structure and incorporation in oxidation-reduction (redox) processes in the most important complex of heavy metals with bacteria structure. Therefore, complex heavy metals can be resistant to washout and have more potential to bioaccumulation. In contrast, when the heavy metals are soluble (raw sludge), the washout rate of them is high and can be accelerated by irrigation [23]. Therefore, the percentage of heavy metals existing in the solid part of the sludge to total concentration of them in the sludge is an important factor for the prediction of bioaccumulation of heavy metals in crops. Prediction of heavy metal content of various groups of foods is important for estimation of total heavy metal uptake in total diet studies. Based on performed studies, main sources of heavy metal uptake are vegetables, fish, and rice. In many developing and undeveloped countries, vegetables are considerable part of people's diet. Thus, it can be a main route of heavy metal uptake. Beside measurement of heavy metal concentration, the health risk assessment was done for determination differences between two groups in produced risk. The health risk assessment and hazard index (HI) through consumption of these two groups of lettuce are summarized in Fig. 2. In this study, we measured the total concentration of mercury (inorganic and organic). Because there is no RfD for the molecular mercury by oral route exposure, thus, the HI was calculated without mercury HQ.

As clear in Fig. 2, the HQ because of consumption of lettuce harvested from farm 2 is almost two times that of the first one. Consequently, hazard index of crops from farm 2 is significantly higher than that of crops harvested from farm 1. This index can illustrate differences in the safety level of application of raw or digested sludge. The induced health risk in lettuce harvested from both farms is too high. Therefore, significant reduction of heavy metal content of raw sludge before digestion is necessary for reduction attributable to health risks. Moreover, there are several methods for heavy metal removal from digested sludge. One of the experienced methods is dissolution of heavy metals in remained water of the digested sludge by decreasing the pH level to 1.5 and removal of heavy metals by sludge dewatering [24]. Recent and novel method consists of using *N*,*N*-bis(carboxymethyl) glutamic acid and citric acid as biodegradable chelators for heavy metal transfer from the solid part of the digested sludge to the liquid ones. This method has high efficiency but it needs long time for chelating [25].

In many health risk assessments, HQ and HI have been calculated to illustrate potential of non-cancer effects. But there are a lot of factors that need to be taken into account in the risk management by the HQ approach. First, HQ was calculated for the 30-year consumption period. Increase of the exposure period can increase the risk to the unacceptable level. Second, the HQ was calculated for the adults and the assumed exposure rates can be unacceptable for the children. Finally, the HQ calculated in the present study is for lettuce as

Table 4 Mean percentage of heavy metal concentration in raw and digested sludge's solids, mg L^{-1} in parentheses and standard deviation in square brackets

Metal	Spring	Summer	Fall	Winter
Raw sludge				
As	0.35 (0.85) [± 0.19]	0.3 (0.87) [± 0.2]	0.35 (0.7) [± 0.23]	0.4 (0.73) [± 0.22]
Pb	0.37 (1.14) [± 0.13]	0.27 (0.93) [± 0.15]	0.36 (0.92) [± 0.16]	0.39 (0.8) [± 0.16]
Hg	0.38 (1.14) [± 0.14]	0.28 (0.94) [± 0.14]	0.34 (0.8) [± 0.17]	0.4 (0.8) [± 0.16]
Cd	0.3 (0.97) [± 0.14]	0.25 (0.75) [± 0.16]	0.37 (0.86) [± 0.18]	0.38 (0.68) [± 0.18]
Digested sludge				
As	0.65 (1.58) [± 0.20]	0.7 (2.05) [± 0.22]	0.65 (1.33) [± 0.18]	0.6 (1.1) [± 0.19]
Pb	0.63 (1.95) [± 0.17]	0.73 (2.53) [± 0.15]	0.64 (1.64) [± 0.13]	0.61 (1.25) [± 0.14]
Hg	0.62 (1.87) [± 0.15]	0.72 (2.42) [± 0.17]	0.66 (1.57) [± 0.16]	0.60 (1.2) [± 0.15]
Cd	0.64 (1.74) [± 0.17]	0.75 (2.26) [± 0.16]	0.63 (1.47) [± 0.18]	0.62 (1.12) [± 0.18]

Table 5 Load rate of heavy metals to the farm 1 and farm 2 and heavy metal accumulation rate in the harvested lettuce

	Raw sludge (or farm 1)				Digested sludge (or farm 2)			
	As	Pb	Hg	Cd	As	Pb	Hg	Cd
Total load (mg)	82.32	93.12	96.64	81.6	65.12	76.8	73.12	68.12
Load rate (mg m^{-2})	9.14	10.34	10.73	9.06	7.23	8.53	8.12	7.56
After harvesting								
Concentration in the top layer (mg kg^{-1})	0.4	0.5	0.58	0.5	0.7	0.85	0.82	0.78
Concentration in 15-cm depth (mg kg^{-1})	0.7	0.83	0.97	0.8	1.1	1.23	1.18	1.14
Harvested lettuce								
Concentration (mg kg^{-1})	1.58	1.65	1.7	1.25	3.1	3.44	3.6	2.85
Bioaccumulation rate (%)	9.2	8.6	8.5	7.5	22	21	23	20

leafy vegetables. As Qureshi et al. mentioned, leafy vegetables have higher rates of accumulation of heavy metals in comparison to the other groups of vegetables [26]. Thus, this study was performed on lettuce. The bioaccumulation rate can be differing for other types of vegetables that can be evaluated in future studies.

Conclusion

The anaerobic digestion of biological sludge can increase heavy metal concentration in the solid part of the sludge. This study shows that application of anaerobic digested sludge can enhance bioaccumulation of heavy metals in the crops in comparison to raw sludge because of keeping heavy metals in organic complex form and release them slowly and make opportunity to the crops for more accumulation of them. This process can increase heavy metal concentration in soil and crops to the unacceptable level depending on their initial concentration in raw sludge.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interests.

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