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Assessing the impact of wastewater irrigation on some toxic heavy metals concentration in grains of Sorghum bicolor

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ABSTRACT

The impact of waste irrigation on the concentrations of the metals Cu, Fe, Ni, and Zn contents in grains of *Sorghum bicolor* was investigated through analyzing (for three seasons 2008-2010) four different-irrigation sorghum grains samples grown on four different-irrigation soil areas according to the experimental design. The two original soils: Soil, historically and experimentally, irrigated with Wastewater (SHEwastewater), and Soil, historically and experimentally, irrigated with wells water (SHEwells water), were analyzed in the 1st season. Wastewater and wells water samples were analyzed in the 1st and 2nd seasons. According to the procedures used in the literature, Samples were collected, pretreated, preserved, digested according to the microwave assisted acids digestion procedures, and analyzed for metals by ICP-AES. Quality control was performed and %R(s) we got were in the acceptable ranges for real samples analysis. Fe contents of the two types of soil samples were not significantly different. Cu and Ni contents of SHEwastewater were higher than that of SHEwells water whereas Zn content of SHEwastewater was lower than that of SHEwells water. In addition, not all metal levels of the two types of soil samples have exceeded the upper EU standards except nickel in SHEwastewater. The average means (of 1st and 2nd seasons) levels of Cu, Fe, Ni of wastewater was higher than that of wells water, but for Zn, the case is reversed. In addition, the average means of all metal ions of both wastewater and wells water was lower than Yemeni standard for irrigation water. Sorghum grains analysis results, for at least two of the three seasons, indicated that: wastewater irrigation resulted in an increase of nickel content of grains of Sorghum bicolor. Wells water irrigation resulted in an increase in zinc content. In addition, there is no significant difference between the effect of wastewater and wells water irrigation on copper and iron contents of grains of Sorghum bicolor.

Keywords: *irrigation*, toxic heavy metals, Analysis, wells water.

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1. INTRODUCTION

In small quantities, certain heavy metals (Chromium, Cobalt, Iron, and Zinc) are a nutritionally essential of a healthy life. Zinc, for example, is a co-factor in over one hundred enzyme reactions. Heavy metals are also common in the manufacture of pesticides, batteries, electroplated metal parts, textile dyes, alloys . . .

etc; many of these products are in our homes and actually add to our quality of life when properly used **[1]**. In the other hand, heavy metals e.g., Cd, Pb, Ni and others are toxic metals, even for those that have bio-importance, dietary intakes have to be maintained at regulatory limits, as excesses will result in poisoning or toxicity **[2]**. Toxic effects and exposure sources of heavy metals are summarized in **Table 1**.

Table 1. Symptoms, toxicity, and Sources of exposure

M	Symptoms and toxicity	Sources of exposure
Cu	Reproductive, hepatocellular, neurotoxicity, dizziness, diarrhea; gastrointestinal bleeding, haematuria, acute renal failure, oliguria, nausea **[3]**.	Sewage sludge and effluent from wastewater treatment plants **[4]**; wire drawing, copper polishing, paint manufacturing, wood preservatives **[5]**.
Fe	Enzyme dysfunction, heart disease, hyperactive behavior, aging, toxic Iron Oxide, chronic infections in our population, cellular poison (cancer) **[6]**.	Occur naturally in soils, which are formed by geological processes **[7]**, Sewage sludge, and effluent from wastewater treatment plants **[4]**.
Ni	Pathological changes of the nasopharynx, hyperplasia, polyploidy rhinitis, hypo-osmium, and anosmia, chronic sinusitis/bronchitis, increased respiratory frequency, nausea, vertigo, pulmonary edema, kidney toxicity, adrenal insufficiency, and death may occur **[8]**.	Occur naturally in soils geological processes **[7]**, mineral processing, paint formulation, electroplating, porcelain enameling, copper sulfate manufacture, and steam-electric power plants **[5]**.
Zn	Impaired immune response, neutropenia, ataxia, lethargy, nausea, vomiting, appetite loss, abdominal cramps and headaches (9); short term metal-fume fever, gastrointestinal distress, nausea and diarrhea **[5]**.	Mining and manufacturing processes **[5]**. Zinc is widely found in nature **[6]**.

In our country, as in many other countries, some farmers reuse chemically- untreated wastewater to irrigate their crops, some of these crops are of human consumption such as grains of Sorghum and wheat. In such countries, the reuse of wastewater in irrigation is still the available solution for problems of wastewater accumulation and Scarcity of natural water for irrigation. The scarcity of surface/groundwater for irrigation is an ever increasing problem around the world, owing to which, use of wastewater in agriculture has become a common reality in the third-fourth of the cities in Asia, Africa and Latin America **[10]**. In the case of soils with poor fertility, it is an important source of nutrients for crop production **[11]**. In the other hand, wastewater irrigation results in an elevation in some toxic heavy metals in soils. These metals moved into the soil, concentrate on the food chain, thereby reaching humans and causing chronic or acute diseases **[12]**. Grains of Sorghum bicolor, in our country, is one of the main human consumption crops, these grains are, sometimes, cultivated by some farmers, depending on wastewater irrigation from the second, and occasionally, from the first pond of the treatment plant. Therefore, the determination of toxic heavy metals contents of these grains is of great importance since these contents, even at traces, they directly enter within meals into a human body and accumulate in body tissues, and, it might reach toxicity levels causing health risks. There are Yemeni standard for a grain of Sorghum **[13]**, Yemeni maximum tolerable limits of toxic heavy metals (mg/kg) wheat flour **[14]**, and WHO upper limits of daily or weekly intake of trace or micronutrient by the human for each **[15, 16]**.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All chemicals and reagents used were analytical reagent grade and used without previous purification, **Table 2** shows chemicals and reagents used.

Table 2. Chemicals and reagents used

Substance	Chemical formula	company	substance	Chemical formula	company
Cupric std. Solution 1000 mg/L	$Cu(NO_3)_2$	Scharlau	30% hydrogen peroxide solution	H_2O_2	
Iron std. Solution 1000 mg/L	$Fe(NO_3)_2$	Scharlau	Hydrochloric acid	HCl	Fluka
Nickel std. Solution 1000 mg/L	$Ni(NO_3)_2$	Scharlau	Nitric acid	HNO_3	SIGMA
Zinc std. Solution 1000 mg/L	$Zn(NO_3)_2$	Scharlau	Sulfuric acid	H_2SO_4	BDH AnalaR
DDW	H_2O	Yemen Star Co.			

2.2. Site description:

Bahira's paddock and Haidrah's paddock were located in Alfallaheen, Ashugiraate farm located south Al-Hawta city in Lahej governorate, Yemen Republic.

2.3. Experimental design and sampling plan

In order to evaluate the influence of the wastewater irrigation on the metallic levels in sorghum grains, four sampling areas were designed as follows:

I- Two sampling areas to be irrigated, experimentally, with wastewater. One of them consisted of a transferred soil, historically, irrigated with wells water for more than 15 years (SHwellsEwastewater), the second consisted of soil, historically, irrigated with wastewater for about 15 years (SHEwastewater). These two areas were designed in Bahirah's paddock in Alfallaheen farm south Al-Hawta city in Lahej governorate, Yemen Republic.

II- Two sampling areas to be irrigated, experimentally, with wells water. One of them consisted of a transferred soil, historically, irrigated with wastewater for about 15 years (SHwastewaterEwells water), and the second consisted of soil, historically, irrigated with wells water for about more than 15 years (SHEwells water). These two areas were designed in Haidrah's paddock in Ashugiraate farm south Al-Hawta city in Lahej governorate, Yemen Republic.

Each designed soil area is 12 m^2 and 15 cm in depth, is designed in order to represent about 0.5 ha of its original soil and it was 2 m far from the surrounding. Each one of the designed four areas was sowed with sorghum grains and frequently irrigated with the kind of water for which it designed.

We designed four different-irrigation Sorghum grains samples: G/SHEwastewater (this sample was grown on Soil, historically and experimentally, irrigated with Wastewater, G/SHwells water Wastewater (this sample was grown on Soil historically irrigated with wells water but, experimentally, irrigated with Wastewater), G/SHwastewaterEwells water (this sample was grown on Soil, historically, irrigated with Wastewater but, experimentally irrigated with wells water and G/SHEwells water (this sample was grown on Soil, historically and experimentally, irrigated with Wells water.

2.4. Sample collection, pretreatment, and preservation

Collection of all samples from location sites were carried out during the period 2008-2010. The collection stage included the process of packaging and labeling for all samples for the period 2008 – 2010 s. The collected samples were adequate for heavy metals analysis. The containers used for holding the samples were polythene containers that are suitable according to the literature **[17]**.

2.4.1. Soil samples

Two soil samples of the two original soil areas were collected and preserved according to recommended methods described in the literature **[18-20]**. After 30 days of sowing, a plastic pipe (0.75-inch diameter, 1-foot length) was used as soil sampler. The collected soil samples were spread on a plastic plate beside the sampling area for partial drying then cleaned from stones and plant resides. The soil samples were homogenized, reduced volume, and sealed in clean polythene containers and preserved in a refrigerator at 4-6 ºC.

2.4.2. Water samples

During the growing period of sorghum, the samples were monthly collected according to method **[21]**; Wastewater samples were collected from different positions in the second pond of the treatment plant, while wells water samples were collected from the output of the pump. The pH was measured within 15 min of collection, then acidified to pH<2 then put in sealed, cleaned polythene bottles and preserved in a refrigerator at 4-6 ºC **[22]**.

2.4.3. Sorghum grains samples

About 125 days of sowing, four sorghum grains (berries) samples were collected and preserved according to the recommended method described in literature **[23]**; 10 berries were collected from 10 cores, then put in paper bags and dried in an oven at below 60 ºC for about 20 h, then the grains were taken from the berries and put in cleaned and sealed polythene containers.

2.5. Instruments and equipment

Smart pH meter SM101M Milwaukee Hanna. A calibrated digital analytical balance, MEETER AE 100, Switzerland (sensitivity 0.0001 g to 100.0 g).

Vista MPX CCP Simultaneous ICP-AES Instrument, Varian. Drying oven with thermostatic control. Microwave digester, Milestone, Microwave Laboratory System, Ethos Touch Control, N. America. Polyethylene containers of different capacities for water, soil, and grains of sorghum bicolor samples. Ceramic mortar and pestle. Polypropylene sieves (1mm, 0.5 mm).

2.6. Samples Preparation and analysis

Sample preparation has to fulfill at least one of the following: making the test sample physically or chemically measurable by the analytical technique; elimination of the interferences; improving the relationship between the amount of analyte and matrix **[23]**. The dry ash digestion method is not suitable for heavy metals determination. With exception of Zn, recoveries using dry ash destruction if compared to microwave method, consistently was too low **[24]**; the method involving HNO_3 acid/H_2O_2 is by far the easiest and fast, at the same time it is reproducible and allows to obtain recoveries comparable with laborious approach **[25]**; losses of Lead and Nickel occur, in open wet digestion, when nitric and hydrochloric acid is used respectively **[26]**. The microwave-assisted digestion procedure leads to accurate results of analysis and could be recommended as a standard approach for plant material samples **[27]**. Ultrasonic assisted acid digestion and a traditional hot plate acid digestion was used for digestion of different samples such as plant samples and the analytical results for heavy metals showed no significant difference at 95% confidence level ($P<0.05$) **[28]**. "For samples with a high organic matter content, such as organic horizons of forest floor layers, plant material, and organic soils, nitric acid digestion could substitute microwave hydrofluoric acid digestion **[29]**.

Microwave-assisted acids digestion procedures were used in this present study for digestion of water, soil and sorghum grains samples.

2.6.1. Soil samples:

Each of the preserved soil samples was homogenized and about 10 g was dried to a constant weight in an oven at below 60 ºC, and then it was ground to fine powder. From this powder, 0.25 g, adjusted to four decimals, was digested with conc. acids (9 mL HNO_3 and 3 mL HCl) in the microwave under pressure programmed for soil sample digestion. The extract solution was diluted to 25 mL with DDW, transferred into polythene bottles and 1-2 h of extraction, the extract solutions were analyzed.

2.6.2. Water samples

From each of the preserved water samples, 45 mL was digested by Conc. Acids (4 mL HNO_3 + 1 mL HCl) with microwave under pressure programmed for water sample digestion. The extract solution was diluted to 50 mL with DDW and transferred into polythene bottles then, 1-2hrs of extraction, the extract solutions were analyzed.

2.6.3. Sorghum grains samples

About 5 g of each preserved sample was dried to constant weight in an oven at below 60 ºC, ground to fine powder, homogenized and then 0.5 g, adjusted to four decimals was digested by a mixture (9 mL conc. HNO_3 and 3mL 30% H_2O_2) with microwave under pressure and program (5.5 min to reach 180 ± 5.0 ºC and continuing at this temperature for 9.5 min). The extract solution was diluted to 25 mL with DDW and transferred into polythene bottles then, 1-2 h of extraction, the extract solutions were analyzed. Quality control, recovery percent (%R) was performed.

3. RESULTS AND DISCUSSION

3.1. Soil Analysis Results

The % recovery (%R) and analysis results of soil samples are shown in **Table 3** The % R(s) were 101 and 111% for Cu and Pb respectivrly.

3.2. Water Analysis Results:

Quality Control as Laboratory reagent blank (LRB) and Sample matrix spike (SMS) was performed. The %R and analysis results of water samples are shown in Table 3.2. The %R for the 1st season were from 90 to 95% for wastewater, 89 to 117% for wells water for Cu and Ni. The recoveries of Fe and Zn were not calculated due to the spiked amount is much lower than their concentrations in the sample matrix.

From **Table 4**, it is very clear that, the average means (of 1st and 2nd seasons) that the levels of Cu, Fe, and Ni in wastewater were higher than that in wells water, 0.0168/0.00700, 1.24/0.152 and 0.0148/0.00785 respectively; (t- test, p=0.000 - 0.02); while Zn is reversed 0.262/ 0.585 (P=0.000).

In addition, the average means of all metal ions of both wastewater and wells water were lower than Yemeni standard for irrigation water.

Table 3. Quality control and analysis results of soil samples, 1st season (winter 2008-2009) and EU standard, 2001.

Matrix / Metal	Q.C Spike 39.95 μg g^{-1}			Results of different soil samples				EU standard [30]
				SHEwastewater		SHEwells water		
	Un spiked matrix (μg g^{-1})	Spiked matrix (μg g^{-1})	%R	Average (μg g^{-1}) n=3	RSD	Average (μg g^{-1}) n=3	RSD	
Cu	43.02	101.3	146	46.43	1.6	43.26	0.9	50 – 140
Fe	3156	NC	NC	3207	0.9	3184	1.4	-
Ni	73.25	111.4	95.5	77.26	0.5	71.80	1.3	30 – 75
Zn	101.0	147.2	116	100.0	0.5	102.2	0.3	150 - 300

NC: not calculated. Because the spiked amount was too lower than the matrix level.

Table 4. Quality control and metal ions levels (mg/L) of wastewater and wells water (mean of 1st and 2nd season), and Yemeni standard for irrigation water mg/L (Ye. std.).

Sample / M	Wastewater Q.C Spike 0.05 (mg/L)			Wells water Q.C Spike 0.05 (mg/L)			Metal ions concentration (mg/L) mean of water samples				Ye. std. (mg/L) (31)
							Wastewater		Wells water		
	Un spiked matrix (mg/L)	Spiked matrix (mg/L)	%R	Un spiked matrix (mg/L)	Spiked matrix (mg/L)	%R	Mean	RSD	mean	RSD	
Cu	0.0146	0.0621	95.0	0.00830	0.0668	117	0.00168	3.1	0.00700	2.1	0.2
Fe	1.363	NC	NC	0.1355	NC	NC	1.24	2.5	0.152	1.7	5.0
Ni	0.0164	0.0612	89.6	0.0117	0.0560	88.6	0.0148	3.6	0.00785	3.8	0.5
Zn	0.344	NC	NC	0.675	NC	NC	0.262	4.5	0.585	2.5	2.0
pH	7.96	7.96	-	7.30	7.30	-	7.96	-	7.30	-	6.5 - 8.4

NC = Not calculated.

3.3. Sorghum grains Analysis

3.3.1. Quality Control

The obtained data were summarized in **Table 5.** This Table showed that the %R(s) in the 1st season was from 87 to 103% for all target metal ions. In the 2nd season, the %R(s) were from 92 to 106 for all metal ions except Cu. In the 3rd season, the %R (s) were from 90 to 93% for all metal ions except iron, its %R was not calculated (NC) because the spiked amount was too lower than matrix level.

Table 5. Quality control for Sorghum grain analysis 1st, 2nd, and 3rd seasons

Sample matrix / Metal	1st season Spike 4.956 (µg g^{-1}) **			2nd season Spike 4.950 (µg g^{-1}) ***			3rd season Spike 0.9810 (µg g^{-1}) ***		
	un spiked matrix (µg g^{-1})	Spiked matrix (µg g^{-1})	%R	Un spiked matrix (µg g^{-1})	spiked matrix (µg g^{-1})	% R	un spiked matrix (µg g^{-1})	spiked matrix (µg g^{-1})	%R
Cu	6.805	11.89	103	5.052	11.96	139	3.484	4.400	93.4
Fe	50.99	55.66	94.2	37.22	46.31 *	91.9	60.17	NC	NC
Ni	1.633	6.153	91.2	1.559	6.816	106	0.9494	1.828	89.6
Zn	46.08	50.39	87.0	37.47	46.96 *	95.9	0.8021	NC	NC

NC= Not calculated.

* = 2nd season Spike for Fe and Zn = 9.891 µg g^{-1}.

** = G/SHwastewaterEwells water

*** = G/SHEwells water

3.3.2. Metal ions levels of Different-irrigation Grains of Sorghum bicolor

From **Fig. 1.** and **Table 6** we can see that, in the 1st season, Cu content of G/SH wells Ewastewater was higher than that of G/SHE wells 4.692/3.529 (p=0.000), and its content of G/SH waste water wells was higher than that of G/SHE waste water 6.805/5.529 (p=0.000), this indicated that experimentally both Wastewater and wells irrigation raised Cu level in sorghum grains. In the 2nd season, Cu content of sorghum grains was lower 4.286/5.052 (p=0.000) due to experimentally wastewater irrigation and was higher 6.615/5.366 (p=0.000) due to experimentally wells irrigation. In the 3rd season, Cu content of sorghum grains was higher due to, experimentally, both wastewater 4.094/3.484 (p=0.001) and wells 5.208/4.541 (p=0.003) irrigation.

Ni content in the 1st season, of G/SH wells, waste water was lower than that of G/SHEwells 0.8750/1.309 (p=0.002), and its content of G/SH waste water wells was higher than that of G/SHE waste water 1.633/1.115 (p=0.02), this indicated that, experimentally, wastewater irrigation lowered Ni level in sorghum grains whereas wells irrigation raised it. In the 2nd season, Ni level was lower 0.7648/1.559; 0.9560/2.792 (p=0.000) due to, experimentally, both wastewater and wells irrigation. In the 3rd season, Ni level was lower due to, experimentally, both wastewater 0.6714/0.9494 (p=0.07) and wells 0.7313/2.622 (p=0.001) irrigation.

Fe level in G/SH wells waste water was lower than that in G/SHE wells 44.01/47.92 (p =0.01), and its level in G/SH waste water wells was higher than that in G/SHE waste water 58.79/50.36, this indicated that experimentally wastewater irrigation lowered Fe level in sorghum grains whereas wells irrigation raised it.

Fe level, in the 2nd seasons, was lower 46.60/53.98; 53.26/61.43 (p=0.000) due to, experimentally, both wastewater and wells irrigation.

Fe level, in the 3rd seasons, was lower 40.03/60.17; 55.14/59.35 (p=0.000) due to, experimentally, both wastewater and wells irrigation.

Zn level in sorghum grains, in the 1st, 2nd and 3rd seasons, was lower due to experimentally wastewater 20.31/28.07, 20.40/37.46, 21.86/39.34 respectively (p=0.003), and was higher due to wells 46.08/18.09, 45.42/23.62, 41.07/27.69 respectively (p=0.000) irrigation, this is maybe because of the higher zinc level in the water of the designed well than that in wastewater.

In addition, from **Table 6**, it is clear that, in the 1st season. Cu of all grains was much lower than the WHO Upper-Level daily intake for the adult person (WHO ULs mg/d.ad). Fe of all grains has exceeded (WHO ULs/day/adult). Zinc of G/SHwastewaterEwell has exceeded (WHO ULs mg/day/adult), whereas of the other grains has not.

In the 2nd season**,** Cu of all grains was much lower than (WHO ULs mg/d.ad). Fe of all grains has exceeded (WHO ULs mg/day/adult). Zn of all grains (except G/SHwaste water wells) has not exceeded (WHO ULs mg/d/adult).

In the 3rd season**,** Cu of all grains was much lower than (WHO ULs mg /day/adult.). Fe of all grains has exceeded (WHO ULs mg/day/adult). Zinc of G/SHwastewaterEwells and G/SHE wells was around the (WHO ULs mg/day/adult) whereas of G/SHE wastewater and G/SHwellsEwastewater was lower.

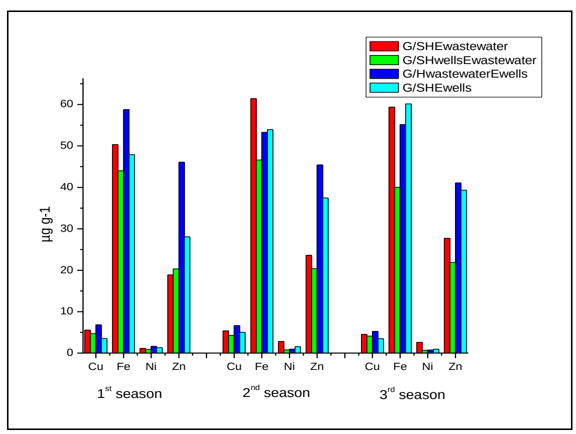

Fig. 1. Metals ions μg g^{-1} of different-irrigation grains of sorghum bicolor 1st, 2nd, and 3rd season.

Table 6. Metal ions levels (μg g^{-1}) of different-irrigation grains of sorghum bicolor 1st, 2nd and 3rd seasons, and WHO (mg/day.adult).

Grains / Metal	G/SHEwastewater			G/SHwells waterE wastewate			G/SHwastewaterEwells water			G/SHEwells water			WHO ULs/d.ad [19-20]
	1st Season	2nd season	3rd season	1st Season	2nd season	3rd season	1st Season	2nd season	3rd season	1st Season	2nd season	3rd season	
Cu	5.529	5.366	4.541	4.692	4.286	4.094	6.805	6.615	5.208	3.529	5.052	3.484	12 – 10
Fe	50.36	61.43	59.35	44.01	46.60	40.03	58.79	53.26	55.14	47.92	53.98	60.17	45 – 40
Ni	1.115	2.792	2.622	0.8750	0.7648	0.6714	1.633	0.9560	0.7313	1.309	1.559	0.9494	-
Zn	18.87	23.62	27.69	20.31	20.40	21.86	46.08	45.42	41.07	28.07	37.46	39.34	45 – 40

4. CONCLUSIONS

Experimentally, wastewater (from Al-Hawtah Lahej treatment plant) irrigation results in an increase in nickel content whereas wells water irrigation results in increases in zinc content of grains of sorghum bicolor.

Experimentally, there is no significant difference between the effect of wastewater and wells water irrigation on copper and iron contents of grains of sorghum bicolor.

Wastewater (as Al-Hawtah Lahej treatment plant) irrigation must not be used for the production of grains of sorghum (as human food) since it raised nickel level in grains of sorghum bicolor.

Al-Hawtah Lahej treatment plant must be maintained and keep management to reduce heavy metals pollution by metal sedimentation.

Conflict of interest

The author declares that there are no conflicts of interest.

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