

Original research

Study of Some Heavy Metals and Protein in Lettuce (*Lactuca sativa*) Irrigated with Sewage-Contaminated Water in the First District of N'djamena, Chad

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Abstract:

This study was conducted during the winter season of 2020 in vegetable farms on the banks of a fiery month, specifically on farms near the drainage canal of the brewery, sailors brush in the first circle, as well as the drainage channel of the brush heater. The study included the evaluation of some heavy metals (Cd^{2+} , Ni^{2+} , As^{5+} , and Cr^{2+}) and plant protein in lettuce (*Lactuca sativa*) irrigated with sewage-contaminated water. The seedlings were planted on December 28, 2020. Follow the sprinkle irrigation system for lettuce seedlings daily. An area of 24 m² (4×6m) was used to plant seedlings. The growth period lasted 56 days. *Lactuca* plant samples from the farm were transferred to the laboratory, of the Chadian Institute of Agricultural Research and Development (ITRAD/LASEP), in sterile plastic bags. The plant sample was washed well with tap water followed by distilled water and dried on the lab's outdoor tables (without exposure to sunlight) for 15 days. Then it was crushed well and sieved with a scale sieve. The test was with a 0.2 mesh sieve per hole. The results of this study showed that the four heavy metal ratios in *Lactuca* sample irrigated with contaminated river water exceed those of the same plant sample irrigated with artesian well water. Highest averages of metals Cd^{2+} , Ni^{2+} , As^{5+} , and Cr^{2+} were 33.53, 0.72, 51.06, 0.953 mg/kg, respectively for contaminated river water *Lactuca sativa* samples. Averages of *Lactuca sativa* samples irrigated with artesian well water were 24.40, 0.44, 24.066, 0.606 mg/kg for Cd^{2+} , Ni^{2+} , As^{5+} , and Cr^{2+} , respectively. So, the studied metals in the *Lactuca sativa* samples exceed the permissible limits of FAO, the China Environmental Protection Agency, European Union, United States Environmental Protection Agency, World Health Organization (WHO) and The Supreme Council for Hygiene of France.

Keywords: *Lactuca sativa*, heavy metals, Plant Protein, N'Djamena, Chad

1- INTRODUCTION

lettuce is one of salad compostae, its scientific name (*Lactuca sativa*), belongs to the astral or compound species (Asteraceae). Lettuce (*Lactuca sativa*) belongs to vegetables that its leaves are eaten fresh, containing many vitamins such as vitamins A, K, C, B, as well as certain minerals such as iron, calcium, magnesium, phosphorus, potassium, as well as fiber (Abdal Rauf et al., 2018)

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Lactuca sativa is a light-loving plant, so its dense cultivation or cultivated in the shade makes it weakly developed, and has disjointed heads that are not integrated with leaves. *Lactuca sativa* lettuce grows in all types of soils and is preferred to be grown in medium-sized soils rich in organic, well-ventilated and drainage materials, with a pH ranging from (5.5-6.6) (Mojahid, 2015). It needs a cold season (climate) influenced by different climate factors. For example, it needs temperature: relatively low. The best temperatures for its growth are 12-18°C by type. The length of the light period also affects lettuce plants, and if the long day prevails, plants rush to bloom, especially in late agriculture, and this effect varies by species *Lactuca sativa* is one of the most important vegetable crops grown in all temperate regions of the world (Salem, 2009)

Lactuca sativa needs a large amount of water, because its root group is superficial, it needs regular irrigation during its various stages of growth, lack of water stops growth and accelerates its bloom, binds the leaves and turns dark green. In the early stages of growth of *Lactuca sativa*, irrigation should be reduced to encourage roots to deepen and spread into the soil, and increased irrigation at this stage leads to weak leaves, yellowing and elongation of the legs. *Lactuca sativa* also needs sufficient amounts of nitrogen fertilizers because it is a paper crop, but beware of adding these fertilizers in large quantities at high temperatures, resulting in the formation of a number of pink pregnant women. (Ghanem et al., 2014). Its growth and production are influenced by many atmospheric and terrestrial environmental factors in which growth is the result of the impact of these factors.

Lactuca sativa is a high source of many vitamins, minerals and antioxidants, perhaps the most famous of which is folic acid and calcium, in addition to its high content of dietary fiber and water (Table 1).

Table (1): Contents of food quantity in *Lactuca sativa* (Mohamed et al., 2008).

Type	food quantity
Calories	15 calories
Carbohydrates	2.79 g
Protein	1.36g
Lipids	0.15 g
Dietary fiber	1.3g
Vitamin A	1405 IU
Vitamin C	9.2 mg
Iron	0.86 mg

Lactuca sativa can cause some potential health risks, as lettuce is a leafy vegetable at risk of contamination as a result of soil or water contamination, including microbiological (Bacterial) contamination. Some improper farming practices may increase the risk of *Lactuca sativa* contamination of certain types of bacteria such as Salmonella and Echerichia coli (E- Coli), which are observed to spread as a result of the irrigation of *Lactuca sativa* farms with contaminated water. As well as heavy metal pollution, *Lactuca sativa* accumulate heavy metals from the soil and to transferred to the human body when ingested, posing a risk to consumer health (Mohamed et al., 2008). The benefits of *Lactuca sativa* can be summarized as follows:

- ▶ Helps the body absorb iron, and prevents foetal deformation, relative to containing folic acid.
- ▶ Increases hemoglobin in the blood, because it contains chlorophyll.
- ▶ Contains vitamin C, which strengthens the immune system
- ▶ Contains antioxidants that rid the body of toxins.
- ▶ Contains 94% fiber and a large percentage of water. So, it softens the stomach. as
- ▶ Its juice helps to activate the stomach and clean the colon.

Heavy metals are a collection of rare elements that are found in small quantities but have toxic effects on plants, animals and humans if found in water, plant or soil at concentrations that exceed critical limits. These minerals are five times denser than water, and have bio accumulation and some heavy metals have function in the body such as copper, selenium and zinc, which are essential for maintaining the metabolism of the human body. Their presence at higher concentrations leads to acute poisoning. They enter the body in various ways, including smelling, digestion or absorption through the skin, and if their penetration rate is faster than their ability to remove them, they accumulate to the risk limit (Abdoul Majid et al., 2010).

Cadmium is transported to food through cigarette smoke and is known to be carcinogenic metal. Like the rest of the metal. Cadmium is transmitted to the body and accumulates even after a long time even in small proportions. Cadmium is associated with soil granules and then dissolves in water and accumulates in plants, fish and animals. (Salem, 2009). Nickel is a heavy metal, with a density (8,902 g/cm³), nickel is the 22nd element in terms of abundance in the earth's crust. Nickel is the 7th element in terms of abundance of transition elements, Nickel may be found in white gold, platinum, and imported jewelry made of yellow and silver gold. Metal may be found in bleaching methods, hair dyeing methods and some foods that contain a high percentage of nickel are dark chocolate, peas... Etc. (Islam et al., 2007). Arsenic is also found in drinking water, especially watery eye water, and prolonged arsenic exposure is one of the causes of cancer of the bladder, lungs, skin, liver and prostate. etc. Organic arsenic compounds are involved in the manufacture of agricultural pesticides, wood conservation and most arsenic compounds dissolve in water. Smoke from the burning of wood residues previously treated with arsenic is a source of it, and then enters the body through breathing (Intizar et al.2002).

Chromium mineral contributes to the process of assimilation (Metabolism) glucose, helps in the production of insulin and regulates blood sugar, and reduces insulin increase. It also lowers cholesterol, lowers weight, increases muscle tissue, helps transport glucose to tissues and has preparations on local markets. It is a necessary element for man (Ousama, 2013). Studies have shown that chromium deficiency reduces the amount of cholesterol and fatty acids used by the liver, which encourages the accumulation of fat in the arteries (Alayyat, 2010).

Heavy metal contamination of soils resulting from irrigation by wastewater is causing major concern due to the potential health risk involved. Disposal of sewage water and industrial wastes is a great problem. Often it is drained to the agricultural lands where it is used for growing crops including vegetables. Wastewater irrigation poses several threats to the environment through contamination by nutrients, heavy metals, salts, and nitrates (Stagnitti *et al.*, 1998). Castro et al. (2009) studied heavy metal content (Cr, Cd, Pb and Hg) in romaine-type lettuce (*Lactuca sativa* L.) cultivated using sewage sludge. Achakzai t al. (2011) studied accumulation of heavy metals (Cu²⁺, Fe²⁺, Mn²⁺, Zn²⁺, Pb²⁺, Ni²⁺ and Cd²⁺) by *Lactuca sativa* irrigated with different levels of wastewater.

This study aimed to determine the concentration of accumulated heavy metals (Cd^{2+} , Ni^{2+} , As^{5+} , and Cr^{2+}) and plant protein in lettuce (*Lactuca sativa*) irrigated with sewage-contaminated water.

2- MATERIALS AND METHODS

2.1. Study site

This study was conducted on vegetable farms on the banks of Chari river, specifically on farms near the drainage canal of the brewery, sailors brush in the municipality of the first district, as well as the drainage channel of the brush heater.

The municipality of the first district is the 4th largest of the capital's 10 districts, with an area of 130,21,000 km² and an estimated population of 125,857 inhabitants and classified as medium-density (Fig. 1). There are larger brush sailors, in which dump their drainage water directly into the Chari River without any treatment. The first district includes 11 lanes. The first circle is geographically located in the border with the city of N'Djamena Fara of the Stone Region of Hadjer Lamis and the municipality of 10th District in the north, south and west of the Chari River, and east of the municipalities of the 2nd and 10nd districts. (This was data in the 1st District of the N'Djamena City Archive/Municipality Secretariat, 2016).

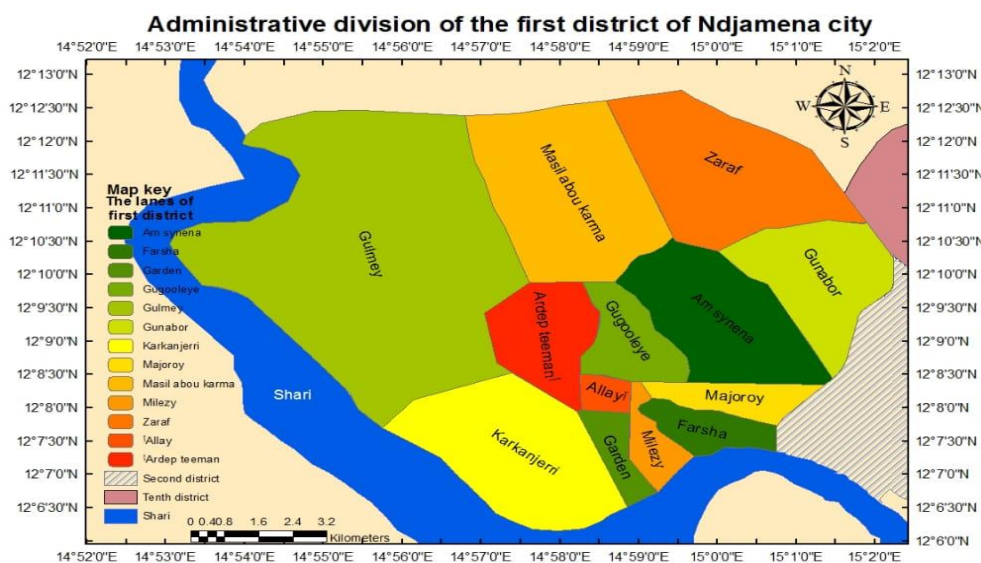


Figure 1. Administrative division of the first district of Ndjamen city. Source: Chadian Tourism Authority, 2020 Study Site - First Circle

2.2. *Lactuca sativa* sampling and analysis

2.2.1. The method of growing *Lactuca sativa*

The seedlings of *Lactuca sativa* were planted on December 28, 2020 with daily irrigation sprinkling irrigation system. An area of 24 m² (4×6m) was used to plant the seedlings. A distance of 3 meters was left to separate the lettuce seedlings irrigated with river water mixed with (untreated) sewage water, and lettuce seedlings irrigated with tap water (the water of the Chadian Water Company's stations). The growth period lasted 56 days and no type of fertilizer (neither natural nor chemical) was added to the lettuce seedlings until they were fully mature.

2.2.2. Prepare *Lactuca sativa* for analysis

Lactuca sativa samples from the farm were brought to the laboratory (water, plant and soil laboratory of the Chadian Institute for Agricultural Research for Development ITRAD/LASEP) on February 06, 2021 in sterile plastic bags. The samples washed well with tap water, followed by distilled water, dried on the lab's outdoor tables (without exposure to sunlight) for 15 days until complete drought, and then weighed the samples were weighed again (the weight of dry samples) and then thoroughly crushed and sieved with mesh 0.2 sieve.

2.3. Chemicals

Chemicals, reagents and standards were of highly pure (Arsenicals Arsenic potassium iodide detector, Cadmium detector (acid1-2- dihydroanthraquinone -3- sodium sulfonate -3- sodium sulfonate), Pure Chrome Detector (chrome chroma) Ver3, Nickel detector (phthalate-phosphate powder, 0.5 ml pan detector solution and EDTA detector (ethylene diamine tetra-acetic acid), Sodium hydroxide solution (10 N), Helianthi detector (green bromocarusol), Phenolphthalein indicator, sulphoric acid concentrated (1N), Extract A (Cr detector +NH₄OH, and Extract C (metal detector + KOH+ EDTA).

2.4. Equipments

Photometer Palintest 5000, Kjeldahl (disarmament and distillation), Sensitive electronic balance (PGWi15i.France).

2.5. Methodology

The ratio of cadmium, chromium, manganese and nickel was determined using the Photometer Palintest 5000. The results of heavy metals (Cd²⁺, Ni²⁺, As⁵⁺, and Cr²⁺) concentration have been expressed in mg/kg (dry material). The concentration of the studied heavy metals have been compared to international standards FAO (2011), (WHO,2013) for food, The Supreme Council for Public Hygiene of France (CSHPF,2009), European Union (EU,2012), United States Environmental Protection Agency (USEPA,1979), and the China Environmental Protection Agency (SEPA,2005) for food.

2.5.1. Determination of Cadmium

50 ml of distilled water was added to 5 tablets (Extract C) in 250 ml measuring flask, and then shake well until complete dissolution. 10 g of the dried lettuce powder was added, shake well for 10 minutes and filtered. 1ml of the filtrate was diluted with distilled water in measuring flask 10ml. One tablet of Cadmecon N0 1S LR Tablet was then added and stirred until dissolution. Another tablet Cadmecon N0 2S LR Tablet was added, and stirred for 20 minutes. Cadmium concentration was measured in the resulted solution at a wavelength of 640 nm. Extract (C) was used as blank in another course in order to estimate the cadmium concentration.

2.5.2. Determination of Chromium

50 ml of distilled water was added to 5 tablets (Extract A) in 250 ml measuring flask, and then shake well until complete dissolution. 10 g of the dried lettuce powder was added, shake well for 10 minutes and filtered. 1ml of the filtrate was diluted with distilled water in measuring flask 10ml. One tablet of the pure chrome detector (chrome Chroma) Ver3 Tablet was then added and stirred until dissolution. Another tablet of the second chrome detector (Chromacol N 4S jR Tablet) was added, and stirred for 20 minutes. Cadmium concentration was measured in the

resulted solution at a wavelength of 640 nm. Extract (C) was used as blank in another course in order to estimate the percentage of cadmium. Cr concentration was expressed by mg/kg.

2.5.3. Determination of Nickel

50 ml of distilled water was added to 5 tablets (Extract C) in 250 ml measuring flask, and then shake well until complete dissolution. 10 g of the dried lettuce powder was added, shake well for 10 minutes and filtered. 1ml of the filtrate was diluted with distilled water in measuring flask 10ml. One tablet of the nickel detector (phthalate-phosphate powder, 0.5 ml for pan detector solution) was then added and stirred until dissolution. Another tablet of the nickelcol N 2S Tablet was added, and stirred for 20 minutes. Nickel concentration was measured in the resulted solution at a wavelength of 640 nm. Extract (C) was used as blank in another course in order to estimate the percentage of cadmium.

2.5.4. Determination of Arsenic

50 ml of distilled water was added to 5 tablets (Extract C) in 250 ml measuring flask, and then shake well until complete dissolution. 10 g of the dried lettuce powder was added, shake well for 10 minutes and filtered. 1ml of the filtrate was diluted with distilled water in measuring flask 10ml. One tablet of arsenic detector (potassium iodide powder) was then added and stirred until dissolution. Another tablet of the arsenic detector (zinc powder and chloride is added) was added, and stirred for 20 minutes. Arsenic concentration was measured in the resulted solution at a wavelength of 640 nm. Extract (C) was used as blank in another course in order to estimate the percentage of cadmium

2.5.5. Plant protein estimate method

0.2 mg of *lactuca sativa* powder was placed in a large test tube with a gelding device. Then add to the sample one tablet (1g) of catalyst and (10 ml) of concentrated sulfuric acid. The test tube was then placed in the Mineraliser for 10 hours until a greenish solution was obtained. The last solution was diluted by adding 50 ml of distilled water. A few drops of phenolphthalein detector and 10 ml of sodium hydroxide solution (10 N) were then added to it until the color changed from green to pink. Then put a test tube in a leather device for distillation. The detailed solution was then taken in a calibration course, and 50 ml of boric acid was added with a few drops of hellenthian detector and green bromocarusol. Distillation was continued until 250-300 ml. The resulted solution containing ammonia was titrated with 1N sulphuric acid in the presence of phenolphthalein indicator. The protein percentage was calculated according to the following equation:

$$\text{Plant protein rate (\%)} = (V_0 - V_1 \times N \times 14 \times 0.001 \times 6.25 \times 100) / \text{PE.}$$

where

V_0 : intial volume of sulphuric acid used in blank titration.

V_1 : The volume of sulphuric acid used in lettuce samples titration.

N: Sulphuric acid normality.

PE: Sample mass.

All measurements made were repeated three times, and each result was expressed in medium terms plus or minus the standard deviation. Statistic analysis program *Recomander* (R×643.2.5. Lnk) was used to carry out statistical analysis of the results, and the confidence level for these results was taken at (P<0.05.)

3. RESULTS AND DISCUSSION

3.1. Concentration of heavy metals in *Lactuca sativa*

The results of heavy metal concentrations (Cd^{2+} , Ni^{2+} , As^{5+} , and Cr^{2+}) in *Lactuca sativa* samples, irrigated with contaminated river water or with artesian well water, were presented in Tables 2 and Figure 2

3.1.1. Cadmium (Cd^{2+})

The average cadmium concentration in lettuce sample irrigated with the river water-mixed with sewage was 33.53 mg/kg, while that irrigated with artesian well water was 24.40 mg/kg. So, the Cd concentration in all the studied lettuce samples (irrigated with contaminated river water or with artesian well water) exceeded the permissible limits issued by FAO; this was attributable to a higher proportion of zinc associated with cadmium, and confirms the results of a study by Gamar (2017).

According to FAO (2011) the maximum percentage of cadmium in lettuce leaves (0.5 mg/kg) is allowed. The Chinese Agency (SEPA, 2005), United States Environmental Protection Agency (USEPA, 1979) and (EU, 2012) identified Cd in fresh vegetables (30 mg/kg). Eating food containing low percentages of cadmium through the food chain causes kidney problems and lung diseases... etc. Because cadmium is characterized by bioaccumulation (Hamid, et al. 2013). Castro et al. (2009) found higher concentrations of Cd in *Lactuca sativa* irrigated with wastewater than in the control.

3.1.2. Nickel (Ni^{2+})

Average nickel concentration in lettuce samples irrigated with river water mixed with sewage was 0.72 mg/kg, while the average nickel concentration for lettuce samples irrigated with artesian well water was 0.44 mg/kg. The cadmium in both samples exceeds FAO's permitted limits. According to (FAO, 2011) the maximum allowable percentage of nickel in lettuce leaves is (0.17 mg/kg). This increase is due to discharge of the wastewater from the beauty salons near the study site. these wastewater contains bleaching, nickel-containing hair dyeing methods. Also, this results from the liquid leaching from a garbage collection site near the river shore that contains food stored for nickel such as beans, peas, lentils... etc. This increase confirms the results of wastewater sample analysis (Gamar, 2017).

Achakzai et al. (2011) studied heavy metal ions (Cu^{2+} , Fe^{2+} , Mn^{2+} , Zn^{2+} , Pb^{2+} , Ni^{2+} and Cd^{2+}) concentrations in lettuce (*Lactuca sativa* L.) planted in soil irrigated with domestic wastewater. The results showed that localities, treatments and their interactions (except Cu^{2+} & Pb^{2+}) generally exhibited highly significant ($P \leq 0.01$) influence on the accumulation of heavy metals. The maximum values of 9.71; 509.06; 32.47; 94.38; 8.58; 5.05 and 7.45 mg kg⁻¹ for Fe^{2+} , Mn^{2+} , Zn^{2+} , Pb^{2+} , Ni^{2+} and Cd^{2+} , respectively, were generally obtained in higher concentration of effluents

3.1.3. Arsenic (As^{5+})

The average nickel concentration in lettuce samples irrigated with artesian well water was 24.06 mg/kg which was within FAO's permitted limits. According to (FAO, 2011), the maximum arsenic rate in lettuce leaves (10-50 mg/kg) is allowed. Average arsenic concentration in lettuce samples irrigated with river water mixed with sewage was 51.06 mg/kg, which was more than the maximum allowed., and this was as a result of discharge to the river the wastewater of from cleaning tools and pesticides used by winers factory and skinny. Exposure to a low level of

arsenic leads to nausea and vomiting with irregular heartbeats and then destruction of blood vessels (Abdul Majid, 2010).

3.1.4. Chrome (Cr^{2+})

Average chromium level in lettuce samples irrigated with artesian well water was 0.953 mg/kg, while the average chromium ratios for well water irrigated lettuce samples was 0.606 mg/kg). All were above the maximum allowed for arsenic in (FAO,2011) lettuce leaves (0.19 mg/kg). This increase is due to the burning of timber residues near the site and smoke from the burning of previously treated arsenic wood is a source of it, in line with (Gamal, 2000). This Cr increase confirms the results of a study (Gamar, 2017).

Table 2: Heavy metals percentages in *Lactuca sativa* samples

<i>Lactuca sativa</i> samples	Heavy metals	Average concentration in dry weight (mg/kg± SD)	<i>P</i> value
<i>Lactuca sativa</i> samples irrigated with Chari river water mixed with untreated wastewater(SICFW)	Cd^{2+}	33.53 ± 2.413	0.0161 *
	Ni^{2+}	0.72 ± 0.130	0.00695 **
	As^{2+}	51.06 ± 5.255	0.0028 **
	Cr^{2+}	0.953 ± 0.130	0.0158 *
<i>Lactuca sativa</i> samples irrigated with untreated artesian well water(SIAWW)	Cd^{2+}	0.953 ± 0.130	0.0161 *
	Ni^{2+}	0.44 ± 0.10	0.00695 **
	As^{2+}	24.066 ± 4.323	0.0028 **
	Cr^{2+}	0.606 ± 0.0850	0.0158 *

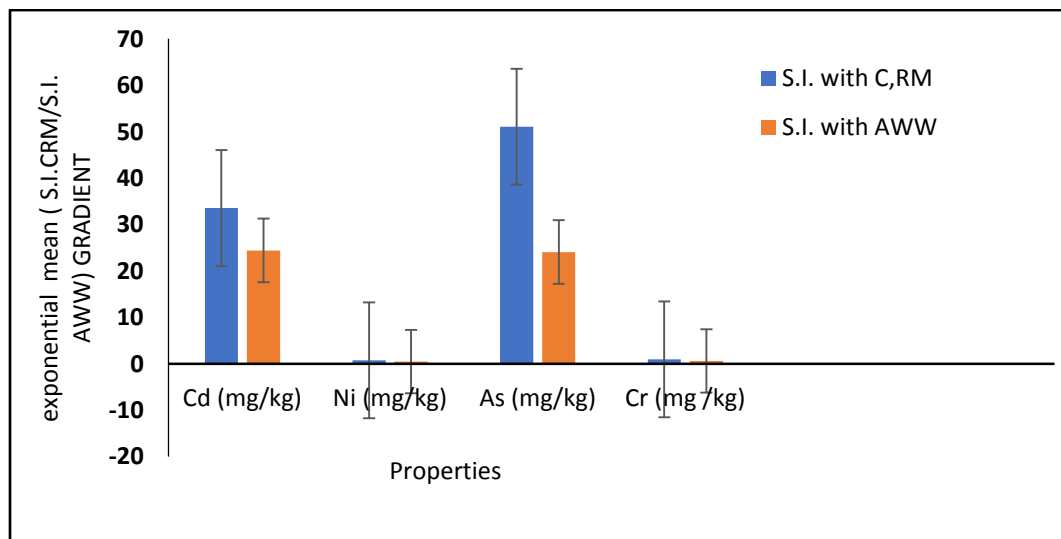


Figure 2: Heavy Metals Ratios in *Lactuca Sativax* Samples

3.2. Concentration of protein in *Lactuca sativa*

The results of protein in *Lactuca sativa* samples were presented in Table 3 and Figure 3. The results in Table 3 and Figure 3 showed a lower in the rate of plant protein in lettuce samples irrigated with contaminated river water (0.98%) than in lettuce samples irrigated with artesian

well water (1.85%) and this value exceeds the optimal limit (1.5 g) of protein in vegetables in general according to WHO standards (Nabil et al. 2017). According to these results, lettuce samples irrigated with artesian well water contain a higher proportion than the maximum allowed in leafy vegetables as per U.S. Environmental Protection Agency (USEPA,2015) standards. This increase in the protein ratio in well water-irrigated lettuce samples from irrigated river water may be due to soil contents of organic matter, water, mineral salts and nutrients, as well as the contents of the river's water mixed with untreated sewage with some chemical pollutants and toxic and harmful elements of lettuce plant (Issawi, 2012).

Table 4: Weight and ratio Vegetarian protein in *Lactuca sativa* samples

<i>Lactuca sativa</i> samples	Weight (g)	Vegetarian protein (%)
<i>Lactuca sativa</i> samples irrigated with Chari river water mixed with untreated wastewater	0.33±1.00E-02	0.98
<i>Lactuca sativa</i> samples irrigated with untreated artesian well water	0.41±1.73E-02	1.85
Control	0.1±0.0	

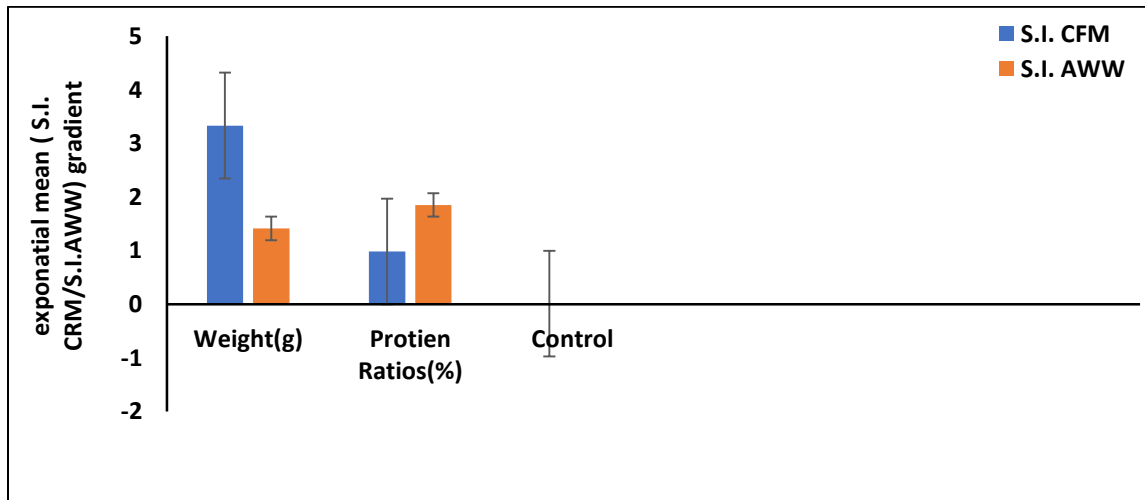


Figure 3: Weight and Protein Ratios in *Lactuca Savtivax* Samples

4. Conclusion

The results of the heavy metal concentrations (Cr^{+2} , Cd^{+2} , Ni^{+2} , As^{+5}) and the plant protein ratio in the lettuce (*Lactuca Savtivax*) plant sample exceeded the permissible limits of the China Environmental Protection Agency (SEPA, 2005), the Supreme Council for Hygiene of France (CSHPF,2009), The Food and Agriculture Organization (FAO, 2011) and the European Union (EU, 2012). Average cadmium for contaminated river water-irrigated lettuce samples, and the average cadmium in lettuce samples irrigated with artesian well water exceed FAO's permitted limits (0.5 mg/kg). Average nickel in lettuce samples irrigated with contaminated river water, and that for lettuce samples irrigated with artesian well water exceed FAO (2011) permitted limits

(0.17 mg/kg). The average arsenic of lettuce samples irrigated with artesian well water is within FAO's permitted limits (10-50 mg/kg). The average chromium ratios for well water irrigated lettuce samples were all above the maximum allowed for arsenic in FAO (2011) lettuce leaves (0.19 mg/kg). The results of the plant protein rate showed a decrease in lettuce samples irrigated with contaminated river water (0.98%) compared to well water-irrigated lettuce samples (1.85%) and this value exceeds the optimal (1.5g) protein content in vegetables in general according to (WHO,2013) standards.

5. Recommendations

This scientific paper recommends the following recommendations:

- 1- The need to treat the wastewater of the winer factories and the skinny wastewater before its use in the irrigation of vegetable farms.
2. The vegetable farm owners should abandon the irrigation of their farms with river water, the results of which confirmed their contamination by increasing the proportions of heavy metals studied.
- 3- The consumer citizens should clean these vegetables with disinfectants (several times and with high compositions) in order to get rid of chemical and biological pollutants from them.
4. The relevant authorities should impose a fine on anyone who uses this untreated water to irrigate vegetable farms and harm the health of the consumer citizen.
- 5-Stakeholders should support farmers' communities around the river by drilling artesian wells for proper irrigation of their farms

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