Investigation of temporal changes in the uptake of heavy metals in strawberries by different organs of rat

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Abstract

In recent years, heavy metal pollution has been shown as a significant environmental issue for human societies. This pollution is caused by industrial and agricultural activities that enter the food chain through different cycles. In this regard, this study aimed to investigate the accumulation and distribution of heavy metals at different times in different organs of rats that were under different nutritional conditions. The experiment was conducted in a completely randomized design with hydroponic strawberry treatments in three replications. Also, different groups of rat (three groups of negative control, polluted and positive control) were fed with strawberries polluted by heavy metals by oral gavage for one, two and three months, every day. The amount of heavy metals in different organs was measured at each stage. 27 rats were studied in this study. Heavy metals decreased the rate of weight gain of rats. Accumulation of these metals was higher in kidney, liver and pancreas than other organs. The brain revealed the lowest accumulation of cadmium (4.78 mg/kg body weight of rat) in the first month among the studied organs. The highest uptake of cadmium and nickel were in the kidney and liver, respectively. In general, results show that the accumulation of heavy metals in the body is a function of time, type of element and organ. If pollution is not controlled in the country's food cycle, these metals are absorbed in large quantities by the body and show their side effects and problems.

Introduction

As a biological point of view, heavy metals are those elements that are strongly absorbed by living tissues, accumulate in them and remove from the tissues with difficulty (Wu et al., 2020). Biologists also consider heavy metals to be elements that are harmful to the life of living things (Shaw, 1989). Heavy metal pollution is one of the major environmental problems and is usually caused by industrial activities such as mining, metal smelting, gas discharge process, energy and fuel production, application of fertilizers, pesticides and municipal waste processing (Ataabadi et al., 2009). Heavy metal pollution in the soil has a detrimental effect on the production and quality of agricultural products, water resources and human health (Thawornchaisit and Polprasert, 2009). The persistence of heavy metals in soil is a threat to human health (Billing et al., 2008). The mobility of heavy metals into the biosphere by human activities is an important process in the geochemical cycle of these metals. It is quite obvious that in urban areas, where various immobile and mobile sources release large amounts of heavy metals into the atmosphere and soil, which is much more than the amount of natural emissions (Tong-Bin et al., 2005). Many studies have been conducted on the effects of heavy metal pollution from industrial and mining activities on soils, plants, water and sediments in different countries, which have shown that areas near to industrial areas have significant air, water and soil pollution. (Kabir et al., 2012; Marrington et al., 1994; Ungaro et al., 2008).

Absorption of cadmium through ingestion is affected by some factors such as age, deficiency of calcium, iron, zinc and protein deficiency, as well as the chemical species of cadmium. Pulmonary absorption of cadmium depends on the size of the suspended particles. After absorption in the body, cadmium participates in metabolic and enzymatic activities and causes disorders in them (Ismaili Sari, 2002;

Huang et al., 2006). Lead toxicity is related to the fact that lead interferes in the normal activity of enzymes. Lead forms strongly bind to free sulfhydryl groups and prevent their function. Lead is toxic to the blood, nervous, urinary, abdomen and reproductive systems. This element also used in animal experiments as a carcinogen and mutagen factor (Pitot and Dragan, 1996; Wani et al., 2015). Lead can accumulate in most parts of the body, but most of it accumulates in the bones, in which case it can be exchanged with calcium, causing bone disorder. Lead in bone can be transmitted to other parts of the body during a fever and cause poisoning. In lead poisoning the sensitive organs are the nervous system, kidneys and bone marrow (Friberg and Nordberg, 1986; Flora et al., 2012). Nickel pruritus is most commonly seen in women due to nickel sources such as the use of jewelry, especially earrings. Prolonged exposure to nickel is carcinogenic and causes cancer of the respiratory system and lungs (Holmes, 1993; Genchi et al., 2020). In addition, nickel poisoning has symptoms such as headache, vomiting and nausea, followed by cyanosis (lack of oxygen to the tissues), fever, and central nervous system disorders (Thobanoglous, 1993).

Yeganeh Shali et al. (2018) also studied the amount of heavy metals absorbed in the body of rat. The researchers showed that the accumulation of heavy metals is a function of the type of element and the body of the mouse. Histological observation of a thin section of rat femur showed that there was a negative relationship between the accumulation of heavy metals and weight, length, area, circumference and maximum bone diameter in rat. The presence of heavy metals in their body indicates that the area was polluted. In a research High levels of alkane phosphatase in the blood of rat studied by Nwokocha et al. (2010) that indicated liver and biliary damage due to consumption of mercury-containing drinking water, which causes to accumulation of this element in the liver. Adding dark chocolate to their diet reduced this effect. In recent years, due to the clarification of the importance of heavy metals in the human body (essential and toxic elements), comprehensive researches have been conducted in this regard, some of them indicate the occurrence of various neurological disorders in the body of rat due to high absorption of zinc (Persson et al., 2003). Disorders of zinc regulation can also cause Alzheimer's disease. Essential elements such as copper, manganese, iron and zinc and non-essential elements such as cadmium, silver, arsenic, aluminum and chromium drastically alter the function of ovum. Many studies in this field have been conducted on rat and have shown that the lack of these elements can reduce or destroy the function and structure of ovum during the growth. On the other hand, the abundance of these elements, even in small amounts, has a negative effect on ovum growth (Hanna et al., 1397). Nunes et al. (2001) examined and measured some morphological parameters such as weight, body length, internal organ weight, and hematological parameters such as blood cell count, hemoglobin concentration, and other factors, and found that kidney and spleen weight in polluted rat is Less than control rat and hemoglobin concentration in the polluted group is higher than the control, which can be due to physiological stress due to exposure to the contaminated environment. Exposure to 2 mg/kg of cadmium after 24 hours caused changes in the level of metatoline protein in the liver, testes, and kidneys of rat (Shimada et al., 2003). Concentrations of lead and cadmium, even in small amounts, cause damage to the liver, kidneys and testes of rodents living near the steel and refined zinc plant (Monika and Katarzyna, 2003). Considering the importance of heavy metals and the damage that these elements have to human health, it is very important to know the changes in the absorption of these elements. Therefore, the aim of this study was to investigate the changes in the uptake of different heavy metals in different organs of rat fed polluted strawberries at different times and treatments.

Materials And Methods

The experiment was conducted as a factorial experiment including two factors of heavy element content in the daily diet at three levels (negative control, polluted and positive control) and period of time at three levels (one, two and three months) in a completely randomized design with three replications.

Cultivation Of Strawberries

Strawberry seedlings of Gavita cultivar were used for greenhouse cultivation. In order to place the plants in the pot and adapt to the new culture condition, they received only irrigation water for two days. Then, until the end of test, the daily feeding of samples were done in drops using the prepared nutrient solution. Each pot received 300 ml of nutrient solution daily through serum embedded next to each pot in six times (every two hours from morning to evening and 50 ml each time). For this study, strawberry cultivar Gavita was added to the diet of rats. Gavita strawberry seedlings were used for cultivation in greenhouse (in February) and the experiment was done in the greenhouse of Bu Ali Sina University of Hamadan under maximum temperature conditions of 25 and minimum 14°C and the crops were harvested in May. Hoagland's nutrient solution for strawberry feeding was prepared according to the method of Hoagland and Arnon (1950) (Tables 1 and 2).

Table 1
Concentration of macronutrients in Hoagland solution

Macronutrients	Concentration (g/l)	Milliliters of stock in 100 liters of solution
NH_4NO_3	91.4	125
$Nah_2po_4h_2o$	35.4	125
K_2SO_4	71.6	125
$CaCL_2.2H_2O$	117.35	125
$MgSO_4.7H_2O$	324	125

Table 2
Concentration of micronutrients in Hoagland solution

Macronutrients	Concentration (g/l)	Milliliters of stock in 100 liters of solution
$MnCL_2.4H_2O$	1.5	125
$NH_4(6MO_7O_{24}.4H_2O)$	0.074	125
$ZnSO_4.7H_2O$	0.035	125
H_3BO_3	0.934	125
$CuSO_4.5H_2O$	0.031	125
$FeCL_3$.6H2O	7.7	125
$C_6H_8O_7.H_2O$	11.9	125

Table 1.

Table 2.

Rats

In order to investigate the effect of heavy metal content in dietary treatments on the amount of metals accumulated in different organs of the body, 27 adult white *Wistar* rats weighing approximately 200–220 g were used. The rats were fed with pellets and also urban water was used to provide the water requirement of the animals. In order to ensure optimal and hygienic storage conditions, the cages of rat were cleaned every two days and fresh food was made available to the rat. The animals were kept for one week to adapt to the experiment environmental conditions. After one week, the studied rats were randomly divided into groups of nine and kept in separate cages.

Food Treatments

The World Health Organization recommends a daily consumption of eight strawberries weighing 18 g (per number) for an adult human weighing 60 kg, which considering the average weight of rats for each of them about 0.5 g of fresh strawberries was taken. The concentrations of heavy metals lead, cadmium, copper and nickel in strawberries were measured by atomic absorption (Figueroa et al., 2008). The mentioned values were used as the basis for preparation for food treatments. The positive control diet included the daily diet of rat plus a certain amount of solution containing heavy metals, this amount of heavy metals was exactly equal to the amount in produced strawberries. To prepare a solution (positive control) containing heavy metals, salts of these metals were used in the laboratory. The required amounts of metals to provide the solution were determined according to the atomic mass of the salts and taking into account the atomic number of each element. Cadmium metal with atomic mass of 228.34 g was used. According to the atomic mass of cadmium (1112.41 g) and the determined amount of this element by atomic absorption in strawberries (7.765 mg/kg), the amount of 15.773 mg of positive control solution was used. Similarly, the salts of lead, copper and nickel were calculated to be 41.560, 9.445 and 28.956 mg/l, respectively. The salts were then poured into a one liter balloon to a volume of one liter and mixed well with a magnetic stirrer. The negative control diet included only the daily diet of rats and did not contain additional content of heavy elements.

Gavage Of Laboratory Rat

In order to prevent the occurrence of lung infection due to the return of excess amounts of solution from the stomach to the esophagus, it was necessary to calculate and regulate the amount of gavage solution. Based on the studies conducted by Mohajel Naibi et al. (2011), the optimal amount of gavage solution was considered equal to 2 ml. After preparing the food treatments along with the daily diet of the rats, 2 ml of the solutions prepared for the positive control and polluted treatments were gavaged to the rats by a special syringe. Also to provide the same nutritional conditions, 2 ml of distilled water was also gavaged daily for the sample of negative control rats, so the rats can be classified into three subgroups:

Group 1: Negative control groups which were gavaged daily by using 2 ml of distilled water.

Group 2: Experimental groups (polluted), which were gavaged daily by using 2 ml of aqueous extract of strawberry.

Group 3: Positive control groups, which were gavaged daily by using 2 ml of a solution which heavy metals were added biased on their salt.

Method Of Conducting Experiments

All animal treatments were strongly in accordance with the national institutes of health guide for the care and use of laboratory animals. The ethics committee for laboratory animal research of Hamadan university of medical sciences approved the experimental protocol (Ethical approval code: ir.umsha.rec.1395.549). At the end of each month, three rats from each cage were randomly selected and weighed. Then deep anesthesia was performed with chloroform until death and they underwent surgery in completely sterile conditions. During surgery liver, kidneys, pancreas, spleen, testicles and brain were removed and placed in a petri dish. The weight of each organ was measured separately and then dried in an oven at 105°C for three days. The dried samples were then ground to a uniform powder. One g of each sample was digested using 10 ml of concentrated nitric acid and 5 ml of hydrogen peroxide (Cobbina et al., 2015). Finally, the concentration of heavy metals in each organ was measured with the help of atomic absorption spectrophotometer model Varian 220 made in the United States.

Calculation Of Percentage Of Adsorption Of Heavy Metals

The percentage of heavy metal uptake by rats were calculated according to the amounts embedded in the diet and the amounts accumulated in the organs of rat (Eq. 1(.

Metal absorption percentage = (amount of metal in organ) / (amount of metal in Diet) × 100 (1)

Statistical Analysis

Experimental factorial including two factors of food treatment (at three levels) and time treatment (at three levels) were analyzed in a completely randomized design with three replications by SAS 9.2 software. The means were compared using Duncan's multiple range tests at five percent probability levels.

Results And Discussion

Copper

The highest mean values of copper content were observed in different organs of the studied rats at the time level of 90 days and the lowest at the time level of 30 days (Table 3). Positive control and polluted treatment compare to negative control treatment showed a higher effect on the uptake of heavy metals in all organs of rats so that the highest content of the element in the organs belonged to the positive control treatment, polluted and negative control respectively ($P \leq 0.05$). In the first trimester, the highest amount of accumulated copper for positive control diet was obtained in the liver and the lowest amount in the rats brains (11.46 and 4.36 mg/kg body weight, respectively). Also, in the third trimester, the highest amount of accumulated copper was calculated for the treatment of polluted food in the liver and the lowest amount in the brain of rats (26.58 and 11.13 mg/kg of rat weight, respectively). Results showed that there was no significant difference in copper accumulation in different organs between the first and second trimesters, but in the third trimester, the kidneys, liver and pancreas received the most amount of copper than other organs. Also, the results showed that over time, the concentration of heavy metals in all studied organs has increased, which can be contributed to the absorption of more metals during the study period and low excretion of elements. In general, the concentration of heavy metals in the body of rats was cumulative and the rate of absorption and excretion was not equal, and this has led to an increase in the concentration of heavy metals. Nutrients antioxidant-containing, such as strawberries, have been reported to affect the bioavailability of heavy metals, toxicity dynamic and their ability to move towards organs, and finally changes their functions in different organs in the body (Adachi et al., 1994; Zhu et al., 1992). Nwokocha et al. (2012) investigated the effect of tomatoes on the accumulation of heavy metals in the body of rat and found that this nutrient reduced the accumulation of heavy metals in various organs of the studied rat because tomatoes contain antioxidant and anti-inflammatory compounds and able to synthesize chelating metal proteins.

Table 3

Compare mean value of copper (mg/kg rat weight) in the different organs and time

Treatment	Time (day)	Brain	Spleen	Testicle	Pancreas	Liver	Kidney
	30	4.36^{e}	5.30^{e}	6.95^{e}	8.42^{e}	11.46^{e}	9.86^{e}
	60	8.17^{d}	9.84^{d}	12.49^{d}	14.29^{d}	18.79^{d}	16.24^{d}
	90	11.13^{c}	13.44^{c}	17.20^{bc}	19.90^{ab}	26.58^{a}	22.94^{ab}
Positive control		12.58^{d}	15.62^{d}	20.88^{c}	23.33^{bc}	30.90^{a}	26.55^{b}
Polluted		9.43^{i}	10.55^{i}	12.50^{h}	15.29^{g}	20.57^{e}	17.98^{f}
Negative control		1.65^{p}	2.42^{n}	3.26^{m}	3.99^{l}	5.36^{j}	4.51^{k}
SEM		0.617	0.217	1.301	0.381	0.769	0.873
Different lowercase letters indicate significant differences at $P < 0.05$							

Table 3.

Cadmium

The results of comparing the mean value of cadmium in the studied organs in Table 4 showed that the highest amounts of elements were at the time of 90 days and the lowest was at the time of 30 days. The highest amount of cadmium accumulated for positive control dietary treatment was obtained in the first trimester in the kidney and the lowest in the rats brain (11.98 and 4.78 mg/kg body weight, respectively). In the third trimester, the highest accumulated cadmium was obtained for the treatment of polluted food in the kidney and the lowest in the rats brain (29.88 and 15.05 mg/kg body weight, respectively). In the case of cadmium, like copper, in the first and second periods, the same intake occurred in different organs relatively. But in the third period, the testicles, spleen, and brain absorbed less cadmium than the other organs including kidneys, liver, and pancreas. In a study conducted by Peterson et al. (1988), rats were exposed to 5 mg/kg of cadmium and then their renal changes were examined, some changes occurred in bones at presence of cadmium, renal negative effect and itai-itai disease that is due to failure and irregularity in kidney function was observed. Yeganeh Shali et al. (2018) also showed that the highest amount of cadmium was accumulated in the kidney.

Table 4
Compare mean value of Cadmium (mg/kg rat weight) in the different organs and time

Treatment	Time (day)	Brain	Spleen	Testicle	Pancreas	Liver	Kidney
	30	4.78^{e}	5.80^{e}	7.25^{e}	8.89^{e}	10.24^{e}	11.98^{e}
	60	12.10^{d}	13.64^{d}	14.91^{d}	17.35^{d}	18.82^{d}	21.73^{d}
	90	15.05^{c}	17.58^{bc}	19.85^{bc}	22.57^{ab}	25.75^{ab}	29.88^{a}
Positive control		20.06^{e}	21.68^{de}	23.60^{cd}	25.62^{c}	29.10^{b}	33.98^{a}
Polluted		9.51^{k}	12.16^{j}	14.88^{i}	18.06^{h}	20.14^{g}	23.57^{f}
Negative control		2.36^{h}	3.19^{mn}	3.54^{m}	5.12^{l}	5.56^{l}	6.05^{l}
SEM		0.339	0.432	0.307	0.486	0.463	1.096
Different lowercase letters indicate significant differences at $P < 0.05$							

Table 4.

Lead

According to the results of comparing the mean value of lead in different organs of rats during the experimental period (90 days) in Table 5, it was observed that the highest amounts of lead at the period of 90 days (end of the third month) and the lowest at the period of 30 days (end of the first month) were observed. The highest accumulated lead was obtained for positive control diet in kidney and the lowest in rat brain (192.27 and 46.07 mg/kg, respectively). Over time, the accumulation of heavy metals in all organs studied has increased significantly.

Table 5
Compare mean value of Lead (mg/kg rat weight) in the different organs and time

treatment	Time (day)	Brain	Spleen	Testicle	Pancreas	Liver	Kidney
	30	46.07^{d}	50.01^{d}	56.08^{d}	62.66^{d}	71.00^{d}	80.47^{d}
	60	93.85^{c}	97.71^{c}	103.70^{c}	113.08^{c}	125.17^{c}	137.55^{c}
	90	125.15^{b}	131.72^{ab}	156.08^{ab}	155.70^{ab}	173.44^{ab}	192.27^{a}
Positive control		131.53^{d}	137.19^{cd}	146.87^{cd}	159.29^{bc}	177.06^{ab}	197.05a
Polluted		112.67^{h}	118.41^{h}	127.81^{gh}	137.82^{fy}	177.06^{abef}	162.14^{e}
Negative control		20.87^{h}	23.84^{m}	27.94^{l}	34.33^{k}	41.60^{j}	51.10^{i}
SEM		1.547	0.916	6.671	7.035	9.089	4.978
Different lowercase letters indicate significant differences at $P < 0.05$							

Table 5.

Nickel

According to the Table 6, which showed the results of comparing the mean value of nickel content in the various organs studied, it can be seen that the highest amount of nickel was observed at the period of 90 days and the lowest at the period of 30 days. The highest accumulated amount of nickel in the third period was obtained in the kidney (equivalent to 33.53 mg/kg body weight of rat) and the lowest amount was obtained in the brain of rats (17.24 mg/kg body weight of rat). Also, in the second period, the highest accumulated amount of nickel was calculated for the treatment of contaminated food in the kidney and the lowest amount was calculated in the rat's brains (22.73 and 12.54 mg/kg body weight, respectively). The reason for the high level of heavy metals in the kidneys can be attributed to its role in the excretion of fluids and increase the circulation of substances in the body of rat. According to researchers, the internal organs of living organisms when they are exposed to heavy metals, constantly accumulate in their bodies and are not decomposed (Nwokocha et al., 2012). Also Lagisz et al. (2005) reported that in treatments exposed to heavy metals after a short period of time; there was still a significant accumulation of heavy metals in their organs tissue even when their dietary intake was reduced. Some reports have shown that constant exposure to heavy metals causes them to accumulate in the body, which related to the kinetics of their absorption and excretion. According to Laskowski et al. (2010), a delay in the physiological response of the body leads to a delay in the elimination or reduction of heavy metals, and as a result, the accumulation of these elements in the body is observed. Chelating of these metals is an effective method to eliminate the toxicity of heavy metals that reduces the mobility and excretion of metal cations

(Graziano et al., 1992). Evidence suggests that differences in diet or metabolism can affect the absorption and excretion of heavy metals (Canuel l et al., 2006). Other studies by researchers on rat and sheep have demonstrated that the tonic acid in green tea has a chelating effect on heavy metals and prevents them from being absorbed by their body tissues (Winiarska, 2013).

Table 6
Results of compare mean value of Nickel (mg/kg rat weight) in the different organs and time

Treatment	Time (day)	Brain	Spleen	Testicle	Pancreas	Liver	Kidney
	30	6.44^{e}	7.57^{e}	8.91^{e}	11.23^{e}	13.26^{e}	15.88^{e}
	60	12.54^{d}	14.18^{d}	15.95^{d}	17.46^{d}	19.97^{d}	22.73^{d}
	90	17.24^{c}	19.33^{bc}	21.68^{bc}	25.10^{ab}	28.99^{ab}	33.53^{a}
Positive control		18.79^{d}	20.54^{cd}	22.90^{c}	26.88^{b}	31.31^{a}	33.67^{a}
Polluted		13.86^{i}	15.71^{hi}	18.21^{gh}	20.94^{fg}	23.64^{f}	29.78^{e}
Negative control		3.57^{n}	4.83^{m}	5.42^{lm}	5.97^{l}	7.27^{k}	8.70^{j}
SEM		0.296	0.418	0.652	0.545	0.785	1.363
Different lowercase letters indicate significant differences at P < 0.05							

Table 6.

Weight Synthesis Of Rat During The Experimental Period

The results of weighting each group at the end of each experimental period are shown in Fig. 1. Negative control rats followed a normal trend of weight changes and the mean weight of each group at the beginning of the experiment, at the end of the first, second and third months were 210.7, 330.7, 343 and 352.7 g, respectively. For the group fed with polluted strawberries (polluted group) equal to 210.7, 291.8, 307.6 and 314.7 g, and for the positive control group equal to 210.7, 262.7, 306.7 and 269.5 g, respectively. As the results show, the three groups showed the highest weight in the first month of weight gain compared to the next two months and this trend gradually slowed down. The most important reason for weight loss in polluted treatment and positive control of heavy metal is accumulation in rat's bodies and disorders of their metabolism. The primary cause of weight loss in all three treatments in the first 30 days can be attributed to the stress of gavage each day and their aging. In contaminated treatment and positive control, in addition to the reasons mentioned, due to the heavy metals in strawberry and heavy metals in their nutrient solution, rats showed nervous stress, therefore, the lowest rate of weight gain was seen in positive control and then polluted treatment, and the highest weight gain was observed in negative control. Persson et al. (2003) investigated the effect of heavy metals on the nervous system of rat and showed that these elements in the body of rat have caused numerous nervous disorders.

According to the codex standard commission, the tolerable cadmium concentration is 0.007 mg/kg body weight per week. Concentrations of more than 200 mg/kg fresh weight can completely destroy kidney function. Nwokocha et al.'s (2012) studied the therapeutic effect of garlic on reducing the accumulation of heavy metals in the liver of rat and results showed that the presence of these metals in the diet decrease the rate of weight gain and even lead causes weight loss. The results of most of these researchers were match with the results of this study. A study on the accumulation of heavy metals in the body of rat in an polluted area showed that the accumulation of heavy metals in the body of rat, in addition to the time of animals in the polluted area (age of animals) and feeding pattern, also depends on the activity of animals. (Wijnhoven et al., 2007).

Figure 1.

Figure 2 shows the weight changes of different treatments in rats per unit time. The rate of weight gain in the first month was faster than the following months. The reason may be related to the body resistance to these metals after daily consumption. In all three groups, the rate of weight gain in the first month showed a higher trend than the next two months and gradually slowed down. The decrease in weight gain rate is related to the content of gavage heavy metals (Fig. 2). In a study by Nunes and Mathias (2001) on the effect of heavy metals on the physical and biological features of the rat body, measured some morphological parameters (such as weight, body length, weight of internal organs) and hematological parameters (such as the number of blood cells) Hemoglobin concentration and other factors and It was observed that the weight of kidney and spleen in polluted rat was lower than control rat and the concentration of hemoglobin in the polluted group was higher than the control due to physiological stress result from exposure to the polluted environment. A study by Cui and Okayasu (2008) on the accumulation of heavy metals in various organs of rat showed that the presence of heavy metals in the diet reduced their t87endency to consume food and, as a result, decreased the rate of weight gain.

Figure 2.

Percentage Of Total Absorption Of Heavy Metals

The percentage of copper uptake by rat fed positive control and polluted diet treatments according to Eq. 1 in three periods were showed in Table 7. Polluted and positive control treatments showed the highest copper accumulation in the first month (51.7% and 85.5%, respectively) compared to the second month (45.7% and 65.6%, respectively) and the third month (42.2% and 63%, respectively). The results indicated that in the Polluted group due to the chemical properties of the copper and its displacement and absorption power, the slope of the graph changed slowly, but in the positive control group due to different conditions and control of pollution of copper adsorption from the first to second month the slope has decreased a lot and then this slope has decreased slowly.

Table 7

Compare mean percentage of element adsorption at different times

Treatment	Time (day)	Ni	Pb	Cd	Cu
Positive control	30	39.84[a]	66.52[a]	40.61[a]	85.50[a]
	60	37.15[a]	63.31[b]	32.51[b]	65.64[b]
	90	33.79[b]	57.28[c]	30.87[b]	63.017[b]
Polluted	30	39.39[a]	58.60[d]	17.83[d]	51.74[c]
	60	26.16[c]	53.15[e]	23.94[c]	45.70[d]
	90	26.36[c]	48.72[e]	20.02[cd]	42.20[d]
Different lowercase letters indicate significant differences at $P < 0.05$					

Table 7 shows the results of cadmium uptake by rats fed different food treatments (positive control and polluted treatment) according to Eq. 1 during the three months of the experiment. Showed the highest accumulation of cadmium in the positive control treatment (40.6%) in the first month compared to the second month (32.5%) and the third month (30.9%). In the second and third months, the percentage of total element absorption decreased compared to the first month. The reason for this can be attributed to the body reaction and excretion of these compounds. In the food treatment fed with polluted strawberries, the highest amount was observed in the second month (23.9%), then in the third month (20%) and the lowest amount in the first month (17.8%).

Table 7.

The results of lead uptake percentage by rats fed positive control and control diet according to Eq. 1 in three periods are shown in Table 7. Polluted and positive control treatments in the first month (58.6 and 66.5%, respectively) showed the highest lead accumulation and in the other two months (53 and 63.3% for the second month and 48.7 and 57%, for the third month respectively) showed a lower percentage of absorption. Table 7 shows the 7% uptake of nickel by rat fed different food treatments (positive control and polluted diet) according to Eq. 1 during the three months of the experiment. Positive control and polluted treatments showed a trend almost similar to the metals in previous sections, and showed the highest accumulation of nickel in the first month (39.8 and 39.4%, respectively) compared to the second month (37 and 26.2%, respectively) and the third month (33.8% and 26.4%, respectively).

In general, according to Table 7 in the positive control and polluted treatments, the highest accumulation of elements was in the first month and higher than the other two months. In the second and third months, the percentage of total metal absorption decreased and reached a constant trend. In fact, there is a decreasing trend in the percentage of elements absorbed by animals over a period of time. It seems that the decreasing process of absorption is directly related to the activation of the immune system of rat and thus reduces the body ability to absorb heavy metals. The percentage of element uptake in the polluted treatment was lower than the positive control treatment throughout the period. The highest reduction in the percentage of element uptake in the polluted treatment compared to the positive control was observed for copper (24.8%), cadmium (14.1%), lead (8.9%) and nickel (6.3%), respectively. The difference in the percentage of studied metals adsorption in different organs of the studied rat in the positive control treatment compared to the treatments fed with polluted strawberries can be attributed to the presence of antioxidant and anti-inflammatory compounds in strawberries. Ascorbic acid in fruits such as strawberries, as one of the most important antioxidant compounds available, somewhat reduces the stresses created by free radicals such as heavy metals (Odriozola et al., 2008). The bioavailability and accumulation of heavy metals in the body depends not only on the biological properties of the body but also on the physical and chemical properties of the heavy metals themselves and the body's ways of dealing with these elements (Neuhauser et al., 1995). To study the daily absorption and excretion of heavy metals by measuring these amounts daily in food and the urine and feces of rat, the researchers concluded that heavy metals are excreted in the urine and feces, and this excretion increases sharply in the eighth week and in the twelfth week peaks and decreases in the sixteenth week. In the studied rats, the daily absorption of heavy metals was 250–250 µg/day, and the excretion of metals through urine and feces was 12.59 and 15–33 µg/day, respectively. About 11–20% of heavy metals are reported to be excreted in the urine or feces, and the remainder is absorbed by the organs and tissues of the body and accumulates there (Cui and Okayasu, 2008).

Conclusion

The produced strawberries in this study were grown in the standard method in greenhouses and compared to method in published horticultural articles. Consumption of agricultural products contaminated by heavy metals poses serious risks to human health. Because daily consumption of strawberries contaminated by heavy metals reduced the weight gain of rat. The lowest accumulation of these elements was observed in the brain. In fact, the liver and kidneys are more vulnerable. Mainly, the cause of metal accumulation in these two parts is related to kidney and liver functions. The liver is responsible for detoxifying and cleansing the body of harmful compounds such as heavy metals. Therefore, the accumulation of heavy metals in this part has increased. Then, liver functions, harmful substances are excreted through the urine or feces. To remove heavy metals through urine, these compounds are sent to the kidneys, and this action increases the concentration of heavy metals in the kidneys. Heavy metals accumulated more rapidly in the first month than in the other two months. The rate of adsorption of these metals in the second and third months was very close to each other. There was a large difference between the accumulation of heavy metals in the treatments fed with polluted strawberries and positive control. This difference is due to the presence of antioxidant and anti-inflammatory compounds in strawberries, but still the accumulation of heavy metals through strawberries during three month was significant. Therefore, the entry of contaminated food such as fruits and vegetables in the human food chain is a serious threat to human health. To control and manage this issue, non-polluted soil should be used to cultivate plants and, chemical fertilizers and pesticides that have the lowest percentage of impurities to feed the plant should be used.

Declarations

Ethical Approval

The subject of plagiarism has been considered by the authors and this article is without problem

Consent to Participate

None.

Consent to Publish

Yes

Authors Contributions

In the order of the authors

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Competing Interests

None.

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Author Contribution Statements

Ghasem Rahimi, Eisa Ebrahimi and Reza Sourati Zanjani: Conceived of the presented idea.

Ghasem Rahimi, Sepideh Yeganeh Shali and Eisa Ebrahimi: Developed the theoretical framework

Meisam Rahimi, Sepideh Yeganeh Shali and Eisa Ebrahimi: Developed the theory and performed the computations.

Ghasem Rahimi, Reza Sourati Zanjani and Eisa Ebrahimi: Verified the analytical methods

Sepideh Yeganeh Shali, Meisam Rahimi and Eisa Ebrahimi: Carried out the experiments

All authors discussed the results and contributed to the final manuscript

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Figures

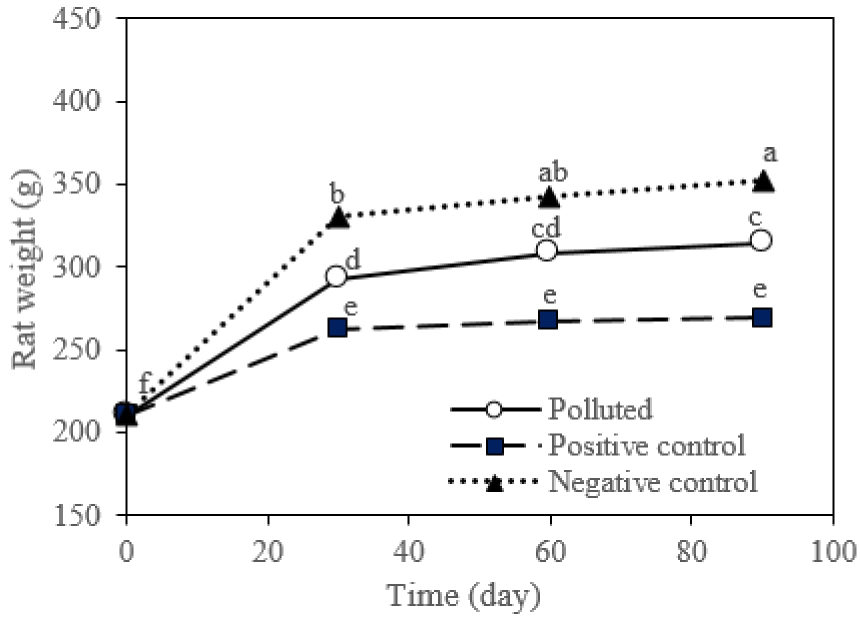

Figure 1

Variation in rat weight during the experimental period Different lowercase letters indicate significant differences at P< 0.05

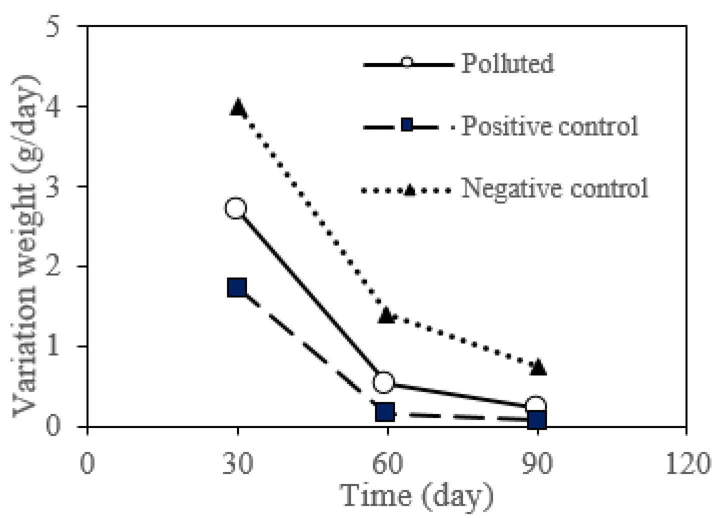

Figure 2

Weight kinetics of rat (g/day) during the experimental period