Interaction between Parsley *Petroselinum Crispum* and Cadmium: Field Study on Some Agricultural Areas in Baghdad City and Experimental Study on Albino Mice Males

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Abstract:
This study consists of two parts, field and experimental. In field part; cadmium concentrations were measured in soil and parsley *Petroselinum crispum* samples of five different agricultural areas in Baghdad city. These areas were located in Al-Rashdiah, Tounis quarter, Al-Shamasiah quarter, Al-Itaifiah and Al-Twaithah. Soil cadmium concentrations were (1.72, 1.25, 1.52, 3.45 and 3.33) mg/kg in Al-Rashdiya, Tunis quarter, Al- Shamasiya quarter, Al- Attaifiya and Al-Tuaithah respectively, while concentrations in parsley were (0.28, 0.26, 0.28, 0.39 and 0.38) mg/kg respectively. Experimentally, five groups of albino mice males (10 mice each) were administered with different concentrations of cadmium (0.0, 0.25, 0.5, 0.75 and 7.5) mg Cd/kg b.w. orally by using gavages needle for 60 days according to 5 days/week protocol, another five mice groups were administered with the same concentrations of cadmium mentioned above and 0.1 ml of parsley *Petroselinum crispum* juice. At the end of the experiment, mice liver and kidney were isolated to measure the bioaccumulation of cadmium.
Keywords: cadmium, parsley *Petroselinum crispum*, bioaccumulation.

التداخل بين المعدنوس *Petroselinum crispum* والكادميوم: دراسة حقلية لبعض الأراضي الزراعية في مدينة بغداد ودراسة مختبرية على ذكور الفئران البيض

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الخلاصة:
تضمنت هذه الدراسة جانبين، حقلي ومختبري. في الجزء الحقلي، قيست تراكيز الكادميوم في عينات التربة ونبات المعدنوس *Petroselinum crispum* لخمسة اراضي زراعية في مناطق مختلفة من مدينة بغداد وهي الراشدية و حي تونس و حي الشماسية و العطيفية والتويثة. كانت تراكيز الكادميوم في التربة (1.72 و 1.25 و 1.52 و 3.45 و 3.33) ملغم/كغم في كل من الراشدية و حي تونس و حي الشماسية و العطيفية والتويثة بالتتالي، في حين كانت تراكيزه في عينات المعدنوس (0.28 و 0.26 و 0.28 و 0.39 و 0.38) ملغم/كغم بنفس الترتيب. مختبرياً، تم تجريع خمس مجموعات من ذكور الفئران البيض مقسمة الى عشرة فئران لكل مجموعة بتراكيز مختلفة من الكادميوم (0.0 و 0.25 و 0.5 و 0.75 و 7.5) ملغم كادميوم/ كغم وزن جسم بطريقة التجريع الفموي الإجباري لمدة 60 يوما بواقع 5 أيام في الاسبوع، وخمس مجموعات أخرى بنفس التقسيم تم تجريعها بنفس تراكيز الكادميوم المذكورة أعلاه و0.1 مل من عصير المعدنوس. في نهاية التجربة عزل الكبد والكلى و قيس التركيز التراكمي الحيوي للكادميوم فيها.

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Introduction:

Cadmium is one of the most toxic heavy metals; exposure to cadmium can occur in the workplace and in the natural environment because of its excessive utilization in industries. It cannot be degraded, therefore; the risk of environmental exposure is constantly increased because of accumulation via the food chain [1]. Cadmium has wide toxic effects which can be resulted in physiological damage to different organs such as liver, kidney, bone, lung, and others [2, 3]. Liver and kidney tissues are the two main targets of cadmium. In low level environmental exposure, about 30-50% of the body cadmium is stored in kidney [4]. For the general population, the main exposure source to cadmium is the food [5].

Cadmium occurs naturally in soils as a result of the weathering of the rocks, anthropogenic sources of cadmium are much more significant than natural ones such as the phosphatic fertilizers which applied extensively to the agricultural soils and atmospheric deposition which is an important source of soil pollution. Other sources include the application of sewage sludge, mining and smelting of zinc sulphide ores [6-9]. Plants take up cadmium by absorption from contaminated soil as well as from deposits on the aerial parts of the plants exposed to polluted air [10], and also during the harvesting processes, storage and/or at the point of sale [10,11]. Leafy vegetables such as lettuce, spinach, potatoes, grains, peanuts, soybeans and sunflower seeds contain high levels of cadmium [9]. For plants grown in the same soil, accumulation of cadmium decreased in the order: leafy vegetables > root vegetables > grain crops [12]. Soil pH and soil type are major factors influencing plant uptake of cadmium from soils [7]. Some plants may accumulate toxic metals at levels which may be harmless to the plant but could be harmful to human if ingested [13].

At the top of the food chain, in animals, cadmium is transported to the liver as a cadmium-protein complex. In the liver, cadmium stimulates synthesis of metallothionein; cadmium-metallothionein is stored in the liver, slowly released from it and moves via the blood mainly to the kidney and other tissues where it can cause damage [5, 14-16]. The maximum levels of cadmium in leafy vegetables, fresh herbs and fungi was determined as 0.2 mg/kg while in liver of sheep, cattle, and poultry 0.5 mg/kg and 0.1 mg/kg in the kidney [17].

Parsley *Petroselinum crispum*, which is a leafy vegetable belongs to the family Umbelliferae (Apiaceae) is known as a rich source of vitamins and minerals especially vitamins C, A and E; iron, calcium, phosphorus and manganese as well as many active chemical compounds. Therefore, it has many medicinal uses, the leaves are diuretic and are giving during the urinary tract infection while the fruit has a diuretic effect too in low doses but higher doses increase the contractility of the intestinal smooth muscles, bladder and uterus. Therefore the fruit is used to remove intestinal spasms, uterus recovery after birth and menstruation complaints, but it could be abortive, so that it is advisable not to be used in medicinal doses during pregnancy [18,19].

Materials and methods:

Study area:

Samples of parsley and soil were collected from five agricultural areas in Baghdad city which export their products to the local markets during September – November, 2011. Those areas, as shown on the map, are Al-Rashdiah, Tounis quarter, Al-Shamasiah quarter, Al-Itaifiah and Al-Twaithah. Figure 1.

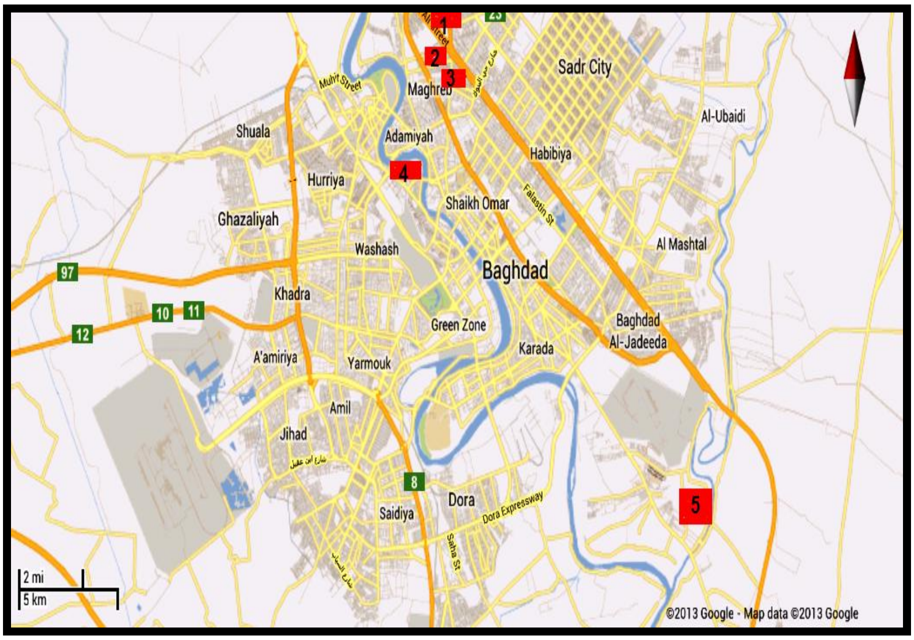

Figure 1- Map of Baghdad city

Soil and plant sampling:

Three random samples of the aerial parts of parsley *P. crispum* and their soil (up to 10 cm depth) were collected from each agricultural area in clean and well closed plastic bags. The plant samples were identified by Prof. Dr. Ali Al-Musawi (Department of Biology, College of Science, University of Baghdad).

Acid digestion of soil samples:

Five hundred grams of soil sample were fully dried in oven at 70 ºC and ground in a porcelain mortar to pass through 200 mesh sieve (Retsch-Germany). One gram of the sieved sample was dissolved in 15 ml of freshly prepared *aqua regia* and left to dry at 60 ºC. The residue was treated with 1.5 ml of concentrated fluoric acid (HF) (BDH-England) and 10 ml of 2 M HNO_3 and left in oven at 60 ºC for 2 hours. The suspension was filtered through a filter paper (Citotest-China). The filtrate was evaporated to 6-8 ml and then diluted to 10 ml with D.W.[20]. Cadmium content of solutions was determined by flameless atomic absorption spectrophotometer (Japan).

Acid digestion of plant samples:

The plant sample was washed with tap water to remove dust and dirt, then washed by D.W. and left to dry at 60 ºC till obtaining a constant weight. The dried sample was ground in porcelain mortar and passed through 200 mesh sieve. One gram of the sieved sample was placed in a muffle furnace (Carbolite-England) for 4 hours at 550 ºC. Three milliliters of 6 M HNO_3 were added to the cinerary sample to dissolve its contents. The solution was filtered and diluted to 20 ml with D.W. [13, 20]. Estimation of cadmium in the aerial parts of parsley was determined by flameless atomic absorption spectrophotometer.

Preparation of cadmium chloride solutions:

Cadmium chloride monohydrate, formula weight 201.33 was used to prepare the different concentrations of the treatment solutions, (0.0, 11.19, 22.39, 33.58 and 335.85) mg of $CdCl_2$ were dissolved in 100 ml of D.W. apart to prepare (0.0, 0.25, 0.5, 0.75 and 7.5) mg Cd^{+2}/ Kg b.w. respectively in each 0.1 ml of cadmium solution.

Preparation of parsley juice:

One hundred and fifty grams of fresh, clean and sliced parsley were mixed with 250 ml of D.W. in a blender and filtered by clean gauze; 0.1 ml of this juice was given to each animal. The juice was freshly prepared every week and kept at 4ºC.

Animals management:

Healthy mature albino male mice at the age of 8-10 weeks and average weight 25 ± 5 g were purchased from the National Center for Drug Control and Research (NCDCR). One hundred male mice were divided randomly into 5 groups of ten animals each (5 animals/cage) in polypropylene cages. The animals were reared and treated at the animal house of Biotechnology Research Center/ Al-Nahrain University at the temperature of 25 ± 5 ºC and 12 ± 2 hours light/day. Diet and drinking water were given *ad libitum.*

Experimental design:

Animals were treated for 60 days orally by using gavages needle according to the protocol of 5 days/week. They were divided into the following groups:

Group (1): control animals, given 0.1 ml D.W. considered as control.

Group (2): given 0.1 ml of 0.25 mg Cd^{+2}/kg b.w.

Group (3): given 0.1 ml of 0.5 mg Cd^{+2}/kg b.w.

Group (4): given 0.1 ml of 0.75 mg Cd^{+2}/kg b.w.

Group (5): given 0.1 ml of 7.5 mg Cd^{+2}/kg b.w.

Group (6): given 0.1 ml of D.W. + 0.1 ml of parsley juice. Considered as a positive control.

Group (7): given 0.1 ml of 0.25 mg Cd^{2+}/kg b.w. + 0.1 ml of parsley juice.

Group (8): given 0.1 ml of 0.5 mg Cd^{2+}/kg b.w. + 0.1 ml of parsley juice.

Group (9): given 0.1 ml of 0.75 mg Cd^{2+}/kg b.w. + 0.1 ml of parsley juice.

Group (10): given 0.1 ml of 7.5 mg Cd^{2a}/kg b.w. + 0.1 ml of parsley juice.

Organ isolation:

The animals were sacrificed by cervical dislocation and dissected to isolate liver and kidney.

Determination of cadmium in tissues:

Liver and kidney were isolated from animals and kept at -20 ºC in clean plastic tubes until digestion.

The frozen organs were allowed to be thawed; 0.5 g of the organ was weighed and placed in a glass beaker, 0.5 ml of diluted HNO_3 (concentrated HNO_3 and D.W. in the ratio 2:1) was added to the tissue and evaporated on a hot plate at 130 ºC for 2 hours until the appearance of white vapors. The solution was filtered and diluted to 25 ml by D.W. [21].

Cadmium content of solutions was determined by flameless atomic absorption spectrophotometer.

Statistical analysis:

Statistical analysis was performed using the statistical analysis system SAS [22]. The least significant difference (LSD) test was used to examine the significant effects of treatments and concentrations on parameter means in this study. P value less than 0.05 was considered as statistically significant.

Results and Discussion:

Cadmium concentrations in soil and parsley:

Table (1) shows cadmium concentrations in both soil and parsley at the study fields. Soil samples demonstrated significant ($P<0.05$) differences among all the fields, where Al-Attaifiya had the highest concentration of cadmium (3.45 mg Cd/kg soil) followed by Al-Tuaithah, Al-Rashdiya and Al-Shamasiya quarter; while Tunis quarter had the lowest concentration (1.25 mg/kg).

Cadmium concentrations in parsley showed less significant differences ($P<0.05$) among the fields. Al-Attaifiya and Al-Tuaithah had the highest values (0.39 and 0.38 mg Cd/kg parsley) with no significant differences ($P>0.05$) between them. Both significantly ($P<0.05$) differ from Al-Rashdiya, Al-Shamasiya quarter and Tunis quarter respectively which showed non-significant ($P>0.05$) decrease in cadmium concentration among them.

Table 1- Cadmium concentrations in soil and parsley *Petroselinum crispum* samples at some agricultural areas of Baghdad city

Agricultural areas location	Cadmium in soil (mg/kg)	Cadmium in parsley (mg/kg)
Al- Rashdiya	1.72 ± 0.03 c	0.28 ± 0.00 b
Tunis quarter	1.25 ± 0.04 e	0.26 ± 0.005 b
Al- Shamasiya quarter	1.52 ± 0.02 d	0.28 ± 0.003 b
Al- Attaifiya	3.45 ± 0.05 a	0.39 ± 0.010 a
Al-Tuaithah	3.33 ± 0.04 b	0.38 ± 0.014 a
LSD Value	0.114	0.026

Different letters in columns refer to significant ($P<0.05$) differences

The results of this study could be attributed to the position of the agricultural areas on the map; because Al- Rashdiya, Tunis and Al- Shamasiya quarters located at the north of Baghdad city; where the population, traffic and industrial wastes are rare compared with Al-Attaifiya and Al-Touaithah. Al-Attaifiya is located near the middle of the city on the western bank of Tigris River; the agricultural land is located in a crowded traffic area, surrounded by scrap landfills which are direct sources of pollution by airborne deposits. Finally, the irrigation system using crude water of the river and the use of industrial fertilizers are additional sources of pollution.

Cadmium occurs naturally in soils, but the anthropogenic activities are much more significant pollution sources [9]. Atmospheric deposition acts as an important source of both soil and plant pollution, which may be originated from fossil fuel combustion, garbage incineration and industrial fume wastes [7]. The phosphate fertilizers contain cadmium; excessive application of such fertilizers to agricultural soils resulted to the addition of 231 tones of cadmium each year in European Union alone [8]. There is no universally accepted safe level for assessing the state of cadmium pollution in soils. Therefore, different levels are used in different countries [23]; but generally, soils contain less than 1mg Cd/kg [14]. Cadmium in soils of thirty countries included Iraq was studied and found that the levels in most studied fields in Iraq did not exceed 0.5 mg/kg [24].

The concentrations of cadmium in parsley are increasing proportionally with cadmium concentration in soils. Heavy metals in plants may be originated from deposits on the surface or absorption from soil by roots, but deposited heavy metals on the surface could almost be eliminated by washing prior to consumption, whereas bio-accumulated metals are difficult to remove [25]. The increasing concentrations of heavy metals in soil increased the crop uptake [26]. Heavy metals accumulation in plants depends on plant species, type of soil and metal, growth stage and the physicochemical parameters of the soil [27]. Leafy vegetables are generally considered to accumulate cadmium to a higher extent than root vegetables and tuberous vegetables [28]. The results of the present study revealed that cadmium in all samples of soil and parsley exceeded the high limits and may form a health hazard for human consumption.

A study to detect cadmium in parsley and other edible vegetables in Sanandaj city- Iran revealed that cadmium concentration in parsley was 0.2 mg/kg dry weight; while the highest concentration was 0.65 mg/kg dry weight in garden cress *Lepidium sativum*. They found that the average concentration of cadmium regardless of the kind of vegetable was 0.3 mg/kg [29]. In some selected cities of Lebanon the contamination level with some heavy metals was studied in 181 vegetable samples included leafy vegetables, over ground vegetables and underground vegetables. In parsley, cadmium concentrations ranged between 0.04-0.55 µg/g dry weight with average concentration of 0.8 µg/g, while the highest average of cadmium concentration (1.25 µg/g) was found in spinach *Spinacia oleracea* [25]. Cadmium average concentration in soil samples of Isfahan –Iran was 1.34 mg/kg while it was 0.17 mg/kg in vegetable samples of the same region. The high concentrations in fruits and vegetables had been attributed to the irrigation water, farm soil, highways traffic and fertilizers [30].

Bioaccumulation of cadmium in the liver and kidney:

Table 2 summarizes the bioaccumulation of cadmium in the liver and kidney of mice treated with different concentrations of cadmium. The results showed a significant ($P<0.05$) increase in cadmium bioaccumulation in both liver and kidney proportional with the increment of cadmium concentration.
In liver, control's mean was 0.165 mg/kg which significantly ($P<0.05$) increased to 0.385 mg/kg at the concentration of 7.5 mg Cd/ kg b.w. Cadmium concentrations in kidney were less than those in the liver of the corresponding treatments. It was 0.135 mg/kg in control and increased to 0.285 mg/kg at the dose of 7.5 mg Cd/kg b.w. which was the highest concentration in kidney.

Table 2- Effect of cadmium different concentrations on cadmium bioaccumulation in the liver and kidney of mice

Treatment	Liver (mg/kg)	Kidney (mg/kg)
Control	0.165 ± 0.002 c	0.135 ± 0.00 c
0.25 mg Cd/kg b.w.	0.183 ± 0.002 c	0.145 ± 0.003 c
0.5 mg Cd/kg b.w.	0.278 ± 0.012 b	0.205 ± 0.015 b
0.75 mg Cd/kg b.w.	0.330 ± 0.011 ab	0.250 ± 0.022 ab
7.5 mg Cd/kg b.w.	0.385 ± 0.018 a	0.285 ± 0.022 a
LSD Value	0.0717	0.0525

Different letters in columns refer to significant ($P<0.05$) differences

Figure-2 shows the interaction effect between cadmium and parsley on cadmium bioaccumulation in mice liver. The administration of parsley decreased cadmium bioaccumulation in liver significantly ($P<0.05$) in the control, 0.75, and 7.5 mg Cd/kg b.w. compared with the administration of cadmium alone.

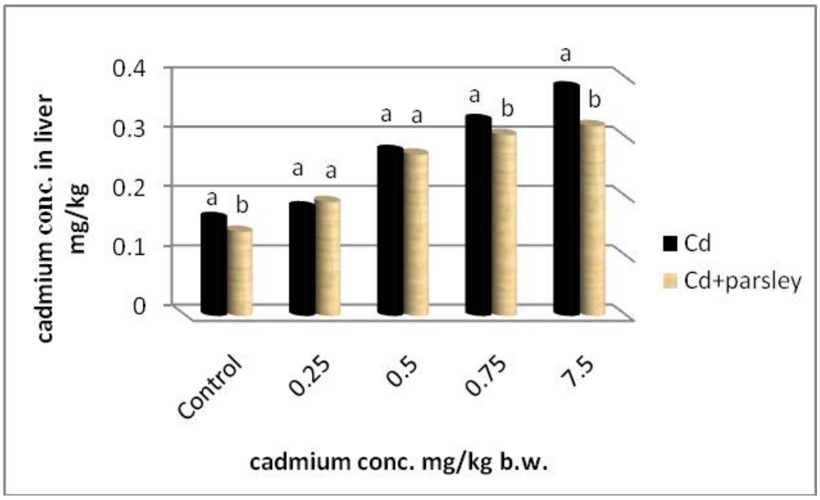

Figure 2- Effect of parsley on cadmium bioaccumulation in the liver of mice treated with cadmium Different letters over columns refer to significant ($P<0.05$) differences

Figure 3 illustrated the bioaccumulation of cadmium among the treatment groups in kidney. The results showed a significant ($P<0.05$) ameliorating effect of parsley on cadmium bioaccumulation in kidney at the concentrations of 0.75 and 7.5 mg Cd/kg b.w. The administration of parsley with 0.25 and 0.5 mg Cd/kg b.w. as well as control groups showed non-significant ($P>0.05$) effects on bioaccumulation of cadmium in kidney.

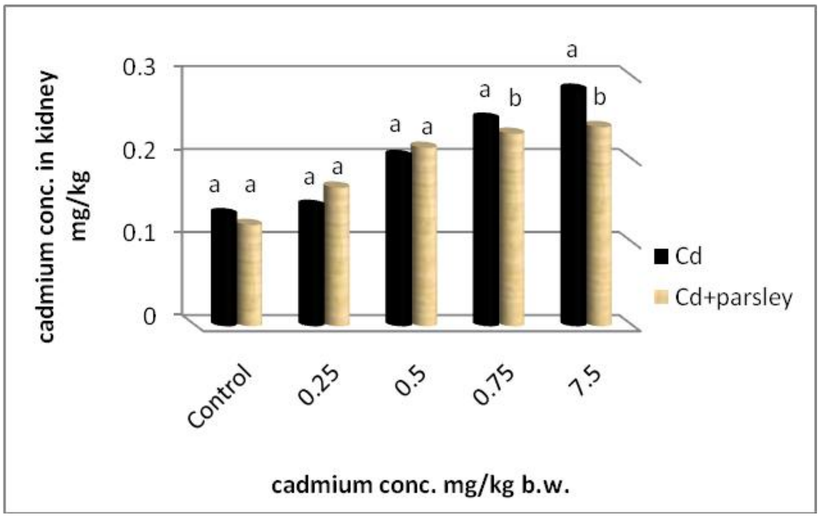

Figure 3- Effect of parsley on cadmium bioaccumulation in the kidney of mice treated with cadmium Different letters over columns refer to significant ($P<0.05$) differences

When cadmium reaches the circulation system due to inhalation or ingestion, it's rapidly cleared from the blood and concentrates in various tissues. Long term of cadmium exposure results in accumulation of the metal mainly in the liver and kidneys as target tissues as well as in other tissues and organs [31]. The severity of organs intoxication depends on the route, dose and duration of the exposure to the metal [32].

The results of the current study showed that cadmium bioaccumulation was higher in liver than in kidney in all treatment concentrations. This may be due to the vital function of liver as the major organ which primarily exposed to toxic and essential substances that enter the body through inhalation or ingestion. Liver extract metals from plasma; metabolizes, stores and redistributes them in various forms either into the bile or back into the blood stream [33].

On the contrary to many other previous studies which demonstrated the kidney as the preferred organ for cadmium bioaccumulation; the present study showed a higher cadmium concentration in liver than in the kidneys. The accumulation of cadmium and other heavy metals in some tissues of two wild rodents, yellow necked mice (*Apodemus flavicollis*) and bank voles (*Myodes glareolus*) were 0.023 and 0.047 mg/kg in yellow necked mice and bank voles liver respectively, while it was 0.071 mg/kg in the kidney of yellow necked mouse and 0.075 mg/kg in the kidney of bank voles [34]. In another study, the concentration of cadmium in the liver and kidney of mice was 10.59 and 6.83 mg/kg respectively which increased after cadmium intoxication to 29.47 and 62.50 mg/kg in the liver and kidney respectively [35].

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