# Lead and Cadmium Contamination in Seeds and Oils of *Brassica napus L* and *Carthamus tinctorius* Grown in Isfahan Province/Iran

*Abbasali Palizban*[*1], *Akbar Badii* [1], *Gholamreza Asghari* [2], *Hossein Mardani-Nafchi* [2]

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## ABSTRACT

**Background:** Lead and cadmium are toxic heavy metals found as major contaminants in food products and edible oils. The aims of this study were to investigate the lead and cadmium contaminations in seeds and extracted oils from Brassica napus L and Carthamus tinctorius grown in the vicinity of industrial sites (Isfahan Zobe Ahan) near Isfahan province/Iran.

**Methods:** In this study, the seeds of B. napus and C. tinctorius were randomly sampled from the farms. The oils of seeds were provided by factory and extracted as well in our laboratory. The two series of washed and unwashed seeds digested with nitric acid and the amount of elements in seeds and oils were measured using GF-AA spectrometer equipped with Zeeman Effect.

**Results:** Cadmium was found in all samples below the Method Detection Limit (MDL, 0.04 µg/L). Lead contaminations were found in all seeds and oils except washed B. napus seeds. The highest levels of lead contaminations were observed in oils of C. tinctorius and B. napus with the amount of 24.74 µg/L and 11.85 µg/L, respectively. The level of lead in unwashed seed oils were significantly higher than washed seed oils ($P<0.05$).

**Conclusions:** The contamination rate of cadmium compared with lead was very low. The higher lead contaminations in unwashed seeds oils compared with washed seeds oils indicated that the contaminant should have been be transferred through the air. Although these observations suggest that the levels of contaminations in edible oils are below the toxic level, long-term exposure may lead to potential health risks.

**Keywords:** *Brassica Napus*, Cadmium, *Carthamus Tinctorius*, Lead, Oils, Seeds.

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## INTRODUCTION

Lead (Pb) and Cadmium (Cd) are two toxic heavy metals found as environmental contaminants, through industrial and agricultural sources and from natural occurrence.

Unfortunately, human activities are the main sources for contamination of water, soil and air.

Pb and Cd are hazardous by inhalation and ingestion and can cause acute and chronic intoxications. These toxic metals are circulated in the environment and can remain in soils and sediments for years. After lead and cadmium are taken up by seeds, they become concentrated through oil extraction processes and finally accumulate in the body of those who consume the contaminated oil. [1, 2]

Although the intestinal absorption of cadmium in is very low (3–5 %) but it can accumulate through the years in the kidney and liver tissues and has a very long biological half-life from 10 to 30 years. Cadmium is highly toxic to the kidney tissue and targets the proximal tubular cells and causes renal dysfunction. [3-7]. Kidneys are also sensitive target organs for Pb exposure which can be due to acute or chronic exposure. The nephrotoxic effects of Pb could occur at very low levels. Low molecular weight proteins freely filter Pb in the glomerulus which is reabsorbed by proximal convoluted tubule cells by endocytosis. Pb causes mitochondrial damage and inside the cells creates high energy free radicals following reduction of glutathione and apoptosis [8, 9].

1. Department of Clinical Biochemistry, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Department of Pharmacognosy & Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
*Corresponding Author: E-mail: palizban@pharm.mui.ac.ir

Contamination of foods and edible oils by heavy metals is one of the main concerns for human health. *Brassica napus* and Carthamus *tinctorius* are widely cultured in many countries due to their high quality oil contents. They are also popular oilseed crops in Iran including Isfahan Province. The proportion of seed oil in some types with suitable conditions of production is 30-40%. These plants successfully grow in regions with low temperature and soils with low fertility [10]. Members of *Brassicaceae* family from genus Brassica are known as heavy metal accumulators. Studies have shown that some of the oilseed rape has the ability to uptake and accumulate Cd from contaminated soil [11,12]. Lead contamination is generally transmitted through air, soil and water. The contamination of lead and cadmium is prevalent in vegetables and herbal medicines. It has been reported that air and water are the most important factors for contamination of vegetables and herbal medicines [13, 14, 15]. With regard to population growth and industrial expansion, hazardous contamination with chemicals and toxic metals is on the increase daily. The sources causing environmental pollutions are different. One of the most important sources are mega-factories which pollute soil, water and air around the agricultural lands. These factories such as iron processing plants are extensively implemented near the farms of the *Brassica napus* and Carthamus *tinctorius* in Isfahan province/Iran. Therefore, identifying such pollutants, determining their effects on population, and preventing the pollution of these resources are crucial. No Study on accumulation of heavy metals in *B. napus and C.tinctorius* and their oils have been reported in Iran. Thus, the current study aimed to determine the concentrations of lead and cadmium in seeds of *B.napus and C.tinctorius* cultivated in the farms within 50-75 km from Isfahan city, in the vicinity of the factories and to compare with lead and cadmium contamination in the extracted oils.

## MATERIALS AND METHODS

### *Sample Preparations*

This study is an observational cross-sectional study. The seeds of *B.napus* and *C.tinctorius* were randomly collected from the farms near an iron processing plant (Isfahan Zoub Ahan) and some other factories during the months of July to November. The seeds and products were collected with the farmers' consent under the authorization of Jahad Keshavarzi & Agriculture Organization, the Isfahan Province/Iran. The seeds of *B.napus* and *C.tinctorius* were kept in the Herbarium of Pharmacy Faculty/Isfahan University of Medicine and were dried and kept in a clean, cool and dark area at room temperature and were divided into two sample series for analysis. The first sample series remained unwashed and the second sample series were washed three times with deionized distilled water and were dried in an oven at 80 °C. The dry seeds were grounded to a fine powder and kept in dark at room temperature for analysis.

### *Oil Extractions from B. Napus Seeds*

The extracted oil from *B. napus* seeds was provided by an oil extraction plant. The seeds of the plant were also from the farms near the Iron Processing Plant (Isfahan Zoub Ahan) and some other factories during the months of July to November.

### *Oil Extractions from C. Tinctorius Seeds*

Two different methods were performed for oil extractions from *C. tinctorius* seeds to find if there is any artificial contamination during oil extraction processing. In the first method or laboratory method, 200 g of grounded *C. tinctorius* seeds were transferred into an Erlenmeyer flask and acetone solvent (200 ml) was added. Erlenmeyer flask was capped and kept in laboratory in a dark place at room temperature for one week, and then the supernatant of digested seeds was separated with a Whathman filter paper No 42 pore size [16]. The filtrated liquid was placed on top of a bain-marie water bath to evaporate the solvent. The liquid at the bottom of the container was *C. tinctorius* oil (CT Oil No.1). In the second method or reflux method, the extraction of *C. tinctorius* oil was briefly as: 200 g of grounded *C. tinctorius* seeds were transferred into an Erlenmeyer flask and then deionized distilled water was added. The flask was placed in a reflux condenser instrument and was boiled at 100˚C. After one hour, the liquid on top was separated and centrifuged with 1500 *g* (*g* is the

relative centrifugal force) for 10 minutes. The oily liquid on top was *C.tinctorius* oil which was separated by Pasteur pipette (CT Oil No.2).

### *Digestion of Seeds*

Two hundred g of grounded powder of *B. napus* seeds was accurately weighed and transferred to a vessel. Five ml of high purity nitric acid (sp gr 1.41 g/ml) was carefully added to the vessel. Acid was added drop wise to prevent foaming. At the end of digestion, 1 ml of high purity 30% hydrogen peroxide was added and then the solution was maintained at 200 °C on hotplate for 20 minutes. The contents of the vessels were diluted to a final volume of 100 ml in a flask with deionized distilled water.

### *Apparatus*

To determine the toxic elements from naturally occurring or production-contamination sources in edible oils a graphite furnace atomic absorption spectrophotometry (GFAAS) was used which is the preferred choice when only a few elements are being analyzed.

The Perkin Elmer AAS Zeeman 3030 was used that was coupled with: graphite oven HGA 600, cooling system, auto-sampler AS 60, 4-fold lamp changer, system-disc with software Rev. 03.01, integrated screen and keyboard and Perkin Elmer Recorder R-100. GFAAS was emplyed due to its selectivity, simplicity, high sensitivity, and its capability for accurate determinations in a wide variety of matrices. The auto sampler cups were soaked in 20% nitric acid overnight to minimize sample contamination, and were thoroughly rinsed with deionized distilled water before use.

**Table 1.** Analytical conditions for analyzing toxic metals in samples.

| Analyte | Pb | Cd |
|---|---|---|
| Wavelength (nm) | 283.31 | 228.80 |
| Slit Width (nm) | 0.7 | 0.7 |
| Lamp Type | EDL | EDL |
| Signal Processing | Peak Height | Peak Height |
| Standard/Sample Volume (µL) | 20 | 20 |
| Read Time (sec) | 3 | 2 |
| Injection Temp (˚C ) | 90 | 90 |
| Calibration Equation Linear | Through Zero Linear | Through Zero Linear |
| Pipet Speed (%) | 40 | 40 |
| Standard Concentration (µg/L) | 0, 20, 30, 40, 50 | 0, 0.5, 1.0, 1.5, 2.0 |
| Automatic Spike Conc. (µg/L) | 10 | 0.5 |
| QC Concentration (µg/L) | 10 | 0.4 |

**Table 2.** Temperature programs for analyzing the lead and cadmium in samples.

| Analyte Steps | Pb | | | Cd | | |
|---|---|---|---|---|---|---|
| **Furnace Program** | **Temp (˚C)** | **Ramp(s)** | **Hold(s)** | **Temp(˚C)** | **Ramp(s)** | **Hold(s)** |
| Drying 1 | 110 | 1 | 20 | 110 | 1 | 20 |
| Drying 2 | 150 | 10 | 10 | 150 | 10 | 10 |
| Drying 3 | 450 | 10 | 20 | 450 | 10 | 10 |
| Pyrolysis | 900 | 10 | 20 | 550 | 10 | 20 |
| Atomization | 1900 | 0 | 3 | 1800 | 0 | 2 |
| Clean Out | 2500 | 0 | 5 | 2500 | 0 | 5 |

**Table 3.** Typical GFAAS Instrument Conditions. Conditions for PerkinElmer 3030 Zeeman AAS with 5100 ZL furnace using end-capped tubes.

| Lead | Cadmium |
|---|---|
| Injection temperature: 100 °C | Injection temperature: 100 °C |
| Wavelength: 283.31 nm | Wavelength: 228.80 nm |
| Slit width: 0.7 nm | Slit width: 0.7 nm |
| Sample Volume: 10 µL | Sample Volume: 10 µL |
| Matrix Modifier: 4 µL 1% $NH_4H_2PO_4$ in 100 µg/mL Mg | Matrix Modifier: 5 µL 1% $NH_4H_2PO_4$ in 100 µg/mL Mg |

### *Reagents and Standards*

All analytical the grade chemicals (AR > 99.0%), $Pb(NO_3)_2$ and cadmium metal were purchased from Merck Chemicals Co., Ltd. (Germany) unless otherwise specified.

### *Stock Solutions and Standards for Pb and Cd*

Lead stock solution (100 ppm) was prepared by dissolving 0.1598 g of lead nitrate, Pb(NO3)2, in a minimum volume of 1+1 nitric acid. Ten mL concentrated nitric acid was added and diluted to 1000 mL with water (1.00 mL = 100 µg Pb).

Cadmium stock solution (100 ppm) was prepared by dissolving 0.1 g of cadmium metal in 4 mL concentrated nitric acid. Then 8.0 mL concentrated nitric acid was added and dilute to 1000 mL with water (1.00 mL = 100 µg Cd). For the analysis of cadmium, calibration standards of 0.5, 1, and 5 ppb were prepared from a 1000 ppm Cd stock solution with 2% analytical grade $HNO_3$. For the analysis of lead, calibration standards of 1, 10, 20 and 40 ppb were prepared from a 1000 ppm Pb solution with 2% analytical grade $HNO_3$. Calibration blank was 2% analytical grade HNO3 solution with deionized distilled water. To check the accuracy of methods, two separate and known standards were used as duplicates for cadmium (0.5PPm) and lead (5 PPM). All glassware used in this study were washed with concentrated $HNO_3$ (1 N) and rinsed with deionized distilled water.

### *Statistical Analysis of Data*

Data analyses were performed using statistical software SPSS-16 with nonparametric Mann-Whitney test.

## RESULTS

Very low levels of toxic elements from seeds and edible oils can be detected with a graphite furnace atomic absorption spectrophotometry (GFAAS). The GFAAS was employed due to its selectivity, simplicity, high sensitivity, and its capability for accurate determination in a wide variety of matrices. Table 4 shows the cadmium and lead contents in seeds and oil products. Cadmium in seeds and edible oils was analyzed following a digestion procedure, and matrix matched standards were used to accurately determine cadmium concentration. The results indicated that cadmium was found in all samples below the Method Detection Limit (MDL) of GFAAS.

**Table 4.** Result for Lead and cadmium analysis from seeds and oils using GFAAS.

| Analyte | Pb (µg/L) | Cd (µg/L) |
|---|---|---|
| *Carthamus tinctorius* seeds (Unwashed) | 0.80 | < MDL |
| *Carthamus tinctorius* seeds (Washed) | 0.75 | < MDL |
| *Brassica napus* seeds (Unwashed) | 0.51 | < MDL |
| *Brassica napus* seeds (Washed) | < MDL | < MDL |
| *Carthamus tinctorius* oil (CT Oil No.1) | 24.74 | < MDL |
| *Carthamus tinctorius* oil (CT Oil No.2) | 21.40 | < MDL |
| *Brassica napus* oil | 11.85 | < MDL |
| Method detection limits (MDLs) µg/L | 0.10 | 0.04 |

The lead contamination in unwashed acid digested seeds was significantly higher than that in washed *Carthamus tinctorius* seeds ($P<0.5$, Figure 1). Figure 1 also shows that lead contamination in the acetone extracted oil by laboratory procedure (Oil No1 CT) compared with extracted oil by reflux procedure (Oil No2 CT) was not significant ($P>0.5$).

The *Brassica napus* seeds were washed and compared with the level of contaminants in unwashed (washed seeds BN & Unwashed seeds BN in Figure 2) and the extracted oil by industrial procedure (Oil BN). Lead (Pb) contaminations were found in all seeds and oils except washed *Brassica napus* seeds. The highest levels of lead contaminations were observed in the oils of *Carthamus tinctorius* (CT Oil No.1, laboratory method), *Carthamus tinctorius* (CT Oil No.2, industrial method) and *Brassica napus* , which were 24.74, 21.40 and 11.85 µg/L, respectively (Figure 3). The level of lead in unwashed seed oils were significantly higher than washed seed oils ($P<0.05$).

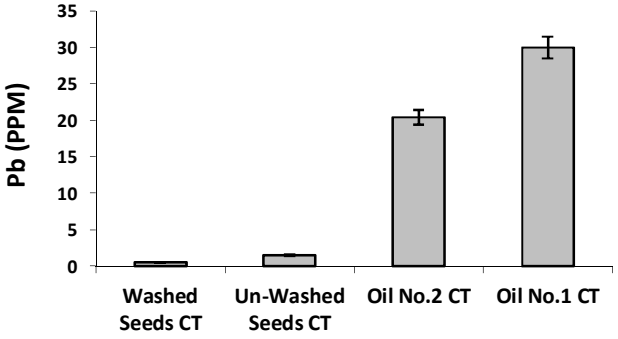


**Figure 1.** Analysis of lead (Pb) in washed and unwashed *Carthamus tinctorius* seeds (washed seeds CT & unwashed seeds CT) and acetone extracted oil with laboratory procedure (Oil No1 CT) and reflux procedure (Oil No2 CT).

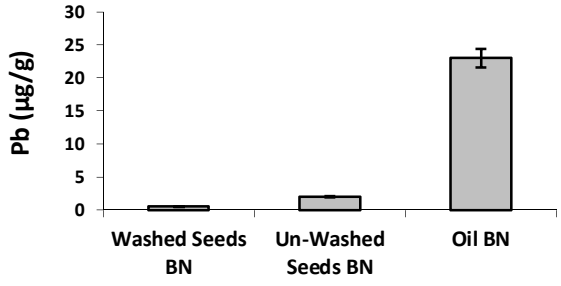


**Figure 2.** Analysis of lead (Pb) in washed and unwashed *Brassica napus* seeds (washed seeds BN & unwashed seeds BN) and the extracted oil by industrial procedure (Oil BN).

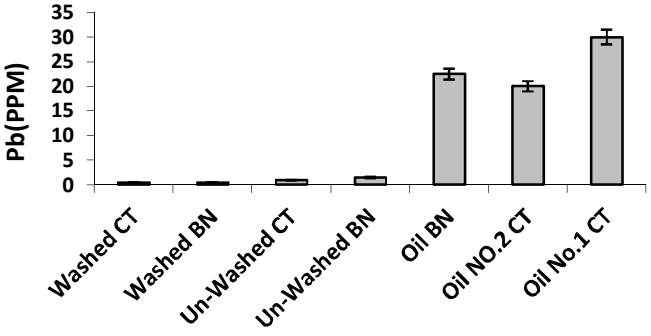


**Figure 3.** Analysis of lead (Pb) in washed and unwashed *Carthamus tinctorius* seeds (washed seeds CT & unwashed seeds CT) and acetone extracted oil with laboratory procedure (Oil No1 CT) and reflux procedure (Oil No2 CT) and analysis of lead (Pb) in washed and unwashed *Brassica napus* seeds (washed seeds BN & Unwashed seeds BN) and the extracted oil performed by industrial procedure (Oil BN).

## DISCUSSION

Lead and cadmium are trace elements that have no physiologic function in humans and plants. They induce various toxic effects in humans at very low doses. The typical signs of lead poisoning are anemia, headache, colic, and convulsions. Long term exposure to lead causes chronic nephritis, brain damage and central nervous system disorders. WHO (1998) approved 10 ppm as the limit for Pb contents in herbal medicines while the dietary intake limit for Pb is 3 mg/week [17, 18]. The main sources of lead and cadmium in atmosphere are urban-industrial emissions. The contaminants emitted by vehicular are also considered to be major contributor for air pollution [19]. Lead concentrations in seed oils in this study were variable with a maximum level of 24.74 µg/L. The contamination in unwashed seeds was higher than washed ones. The results indicated that the lead present in oil seeds could have been transferred from air following absorption by plants. In this regard, it has been reported that the difference in lead concentration between washed and unwashed samples in plants such as *Salvia officinalis* is significant. In fact washing of seeds helps to remove the contaminants if physicochemical absorption of heavy metals accrues in plants [20, 21].

Cadmium is a toxic heavy metal with very low absorption level in humans (3-5%) after exposure with contaminated foodstuff. Cadmium accumulates in human body and damages mainly the kidneys and liver. Cadmium is retained in human body specifically in liver and kidney with a long biological half-life (10-30 years). The lowest level of lead which can cause yield reduction is 5-30 ppm, while the maximum acceptable concentration for foodstuff is around 1 ppm [20]. Graphite furnace atomic absorption spectrometer is capable of having enough sensitivity (based on peak area) of 30 pg/kg for lead at 283.31 nm wavelength and 1.3 pg/kg for cadmium at 228.80 nm wavelength.

Cadmium contamination in the samples was very low. Previous studies indicated that air pollution has little effect on cadmium concentration in the region of the sampled plants [22]. The cadmium concentration in ten plants in Egypt was reported to be 0.05-0.3$\mu g.g^{-1}$ [23]. A study in Pakistan reported that the medicinal plants (n 24) contain cadmium in a range of 0.05 to 12.06 $\mu g.g^{-1}$ [24]. In Iran it was also reported that cadmium contaminations in vegetables grown around Sanandaj City was 0.2-0.65 $\mu g.g^{-1}$ [25]. Another study in Turkey also reported that the cadmium amount in vegetables was 0.24-0.97 $\mu g.g^{-1}$ [26]. Several studies reported that cadmium concentration in vegetable was within safe limit [16,27]. Joint Expert Committee on Food Additive of FAO and WHO offers the authorized index of provisional tolerable weekly intake (PTWI) for each one of the metals. This index is 60$\mu g.day^{-1}$ per 60 kg body weight (kgBW) for cadmium [17, 18]. Estimates of daily consumption of oil seeds indicate that the obtained concentration of cadmium was less than the declared authorized index. Special attention should be paid the cadmium level in medicinal plants because in some parts of the world the rate of contamination is high. Humans receive about 60% of authorized PTWI of cadmium through food and water [27] and people with renal failure are authorized to consume at most 50% of offered PTWI [16]. In a study, concentration of metals (aluminum, arsenic, lead, and cadmium) was examined using atomic absorption spectrophotometry in medicinal plants including: *Thymus vulgaris, Melissa officinalis, Achillea millefolium, Rosmarinus officinalis, and Salvia officinalis* around Arak city/Iran. The minimum and maximum levels of toxic metals in these plants was reported to be

3.022$\mu g.g^{-1}$ and 0.254$\mu g.g^{-1}$ for lead and 0.031 $\mu g.g^{-1}$ and 0.144 $\mu g.g^{-1}$ for cadmium, respectively [28]. Fortunately, in the present study the cadmium content in seeds and oils was lower than WHO recommended levels in foodstuff. It seems that air and soil in the region of our study is very rarely contaminated with cadmium.

## CONCLUSION

Lead contamination rate in the plants in the present study could be related to the presence of an iron processing plant in 20-50 km distance from cultivation sites of the plants. In view of the findings, washing oil seeds is effective in lowering surface contamination. If the edible oils were consumed for short-term and in low amounts, these seeds could not be hazardous due to the lower absorption than that of authorized amount of cadmium and lead through air. However, it is necessary to have complete washing steps for seeds prior to tapping oil. Although edible oils extracted from the seeds cultivated in the region under this study had very low levels of lead and cadmium, long-term use of these oils should be avoided.

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