Advances in Bioresearch
Adv. Biores., Vol 16 (6) November 2025: 451-456

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.6.451456

Advances in Bioresearch

ORIGINAL ARTICLE

Analysis of Bioaccumulated Heavy Metals in *Eruca sativa* Seed Oil in Western Rajasthan

Prakash Ram, Praveen Kumar, Bharat Singh and Sangeeta Parihar*
Department of Chemistry, Jai Narain Vyas University, Jodhpur 342005 (Rajasthan), INDIA
*Mail Id: sp.ch@jnvu.edu.in

ABSTRACT
Eruca sativa seed oil from Rajasthan's desert region was examined for fatty acid composition, chemical properties, and heavy metal concentration (ppm). Heavy metals from the seed oil of Eruca sativa are analysed using MP-AES. Seed oil contains the following heavy elements in ppm order: Zn (1.69), Fe (0.23), Pb (0.09), Cu (0.03), Mn (0.03), Ni (0.01), Co (0.01) and Cr (0.01). Oil from Eruca sativa was examined in terms of its physical and chemical characteristics. A detailed investigation revealed 37.5% oil content. The fatty acid composition of oil sample was determined using High-Performance Liquid Chromatography (HPLC). Total fat content consisted of 9.81% saturated fat, 70.20% monounsaturated fat, and 19.40% polyunsaturated fat. The highest concentration of fatty acids was 42.38% linoleic acid, which was followed by erucic acid (42.60%), oleic acid (19.40%), linolenic acid (12.40%), palmitic acid (8.40%), and stearic acid (5.62%). Small quantities of stearic acid (1.41), eicosadienoic acid (0.40), and palmitoleic acid (0.10) were isolated.
KEYWORDS: *Eruca sativa, Fatty acid, Heavy metals, MP-AES, HPLC*

Received 20.08.2024 Revised 17.10.2024 Accepted 28.11.2025

How to cite this article:
Prakash R, Praveen K, Bharat S and Sangeeta P. Analysis of Bioaccumulated Heavy Metals in *Eruca sativa* Seed Oil in Western Rajasthan. Adv. Biores., Vol 16 (6) November 2025: 451-456.

INTRODUCTION

In some regions of the Middle East, India, and Pakistan, *Eruca sativa*, also known as taramira, is planted as a minor oil crop and used to make various traditional remedies and medicines [1]. It is a member of the Brassicaceae family of plants. The medical benefits of taramira include being an aphrodisiac, diuretic, astringent, digestive, tonic, laxative, anti-inflammatory for colitis, and having rubefacient qualities. [2, 3]. In the arid regions of north-west India (Rajasthan, Haryana, Punjab, MP, and UP), where it is commonly grown along with gram and barley, taramira is a herbaceous annual that grows 2 to 4 feet tall [Fig. 1]. It doesn't require a lot of preliminary tillage because of its efficient and fast-penetrating root structure, which allows soil water to be expelled from deep soil layers. It is a resilient crop that can be successfully grown in arid regions and poor, sandy soils with retained moisture during years of extreme drought combined with late rabi rains; it is the sole choice for planting on soils with little moisture availability. [4]
A significant portion of India's oilseed output comes from Rajasthan. The oil is quite bitter and smelly after extraction. Although taramira oil may be used to create a mustard-like substance, the pungency is different from that of mustard oil. [5, 6] Before using the oil for pickling, salad dressing, or cooking, it is stored in India to reduce its acridity. The oil is also used on the skin as a moisturizer and for rubs. [7] Animal feed can also be made from the seed cake, a byproduct of the oil-making process. Up to 35% of oil and 27% of protein may be extracted from the seeds. [3, 8, 9] Taramira oil and cake have not yet found a market. The oil is unlikely to find a market in the food business because of its unpleasant smell. Therefore, it would be perfect if it could be transformed into an environment-friendly fuel for the transportation sector. [10].
Due to its endurance and adaptation to difficult climatic conditions, *Eruca sativa* is favoured above other comparable species as an important marginal crop planted on land with low fertility [11-15]. There were available Eruca lines with larger, yellower seeds as well as better plant and seed yields [16]. Its seed oil has nutritional value for people, therapeutic and aesthetic benefits, and is used as a lubricant. [17, 18] Its

system of genetic transformation and regeneration, Eruca might be made into a safe industrial oil crop due to their resistance to salt, powdery mildew, and stem rot, as well as its limited crossability with the edible oilseed rape. [12,19-23] Soil and water naturally contain heavy metals, including cadmium, copper, nickel, zinc, chromium, and lead or that are contaminated due to human activity can induce bioaccumulation that has an adverse effect on the entire ecosystem and represent a risk to the health of all life forms [10]. The objective of our study was to evaluate the effects of heavy metals—cadmium, nickel, zinc, lead, chromium, copper, and mercury—on germination of *Eruca sativa* seeds, their early seedling growth, and the potential use of Eruca in the phytoremediation of heavy metal-contaminated soils.

FIGURE 1: IMAGE OF SEEDS AND PLANT OF *ERUCA SATIVA*

MATERIAL AND METHODS

SAMPLING:

Eruca sativa seeds were gathered from the Nagaur region of western Rajasthan, and extracted once the fruits had been sun-dried. After being washed and deshelled, the seeds were dried for 35 minutes at a temperature of 100 to 110°C. Before being extracted, the seeds were ground up in a grinder.

REAGENTS:

Superior grade chemicals included HF, $HClO_4$, H_2O_2, HNO_3, H_2SO_4, and H_2O_2. All dilutions utilised double deionized water. Before being used, the entire set of glassware and plastic was cleaned by soaking it in diluted HNO_3 and then washing it with purified water. The heavy metals (Pb, Cd, Zn, Cr, Cu, and Ni) required for calibration were made into working standard solutions by diluting a stock solution containing 1 mg/L.

EXTRACTION OF OIL

The oil from the powdered seeds of *Eruca sativa* was extracted using a Soxhlet extraction method with mild petroleum ether (40–60°C). In accordance with the instructions of the American Oil Chemists' Society (AOCS), the solvent was completely removed by using a rotary evaporator under vacuum conditions.[24] Transesterification was used to produce methyl esters from the oil [25], and various tests were conducted to check for the presence of any unusual fatty acids, including direct analytical TLC analysis [26], 2,4-DNP TLC analysis [27], picric-acid TLC analysis [29], the Halphen test [28], and alkaline picrate analysis [30].

DIGESTIVE PROCESS OF SEED OIL:

This experiment was conducted in a 100 ml Pyrex glass beaker. A beaker containing seed oil and concentrated HNO_3 was maintained for 24 hours at room temperature to ensure cold digestion of mixture. The mixture was then heated to 50°C for four hrs and subsequently boiled in a 1:5 HCl and HNO_3 solution to decompose any organic materials. Using deionized water, the solution volume was adjusted to 25 millilitres after cooling and filtering.

MINERAL METAL ANALYSIS:

Atomic Emission Spectroscopy is a technique utilized to evaluate both the overall quantity and the speciation of heavy metals at their environmental concentrations. The process is straightforward and quite precise. In this study, we used the MP-AES technique to measure the amount of heavy metals present in oil of *Eruca sativa* seeds. To extract Pb, Cd, Cr, Ni, Zn, and Cu from a unique aqueous biphasic

system, the hydrophobic salt of Tetra hexyl ammonium bromide was added without the use of salting-out agents or organic solvents. Utilizing the chemical 4-(2-pyridylazo)-resorcinol, the recovery of the metal ions was quantitatively. The extract was added straight to an MP-AES apparatus after being diluted with ethanol/HCl. The MP-AES detection settings and the variables that affect extraction (concentration of the reagent, pH, length of phase contact, etc.) were examined and refined.

PREPARATION OF STANDARD FOR METAL:

Our focus is on spectrophotometric measurements of solutions that contain extremely low concentrations of metals. To conduct this analysis, a very low concentration of the specific metal must also be present in the standard solution. HCl, HNO_3 (in a ratio of 1:3) and one gram each of cadmium, nickel, iron, lead, and zinc are mixed with deionized water to prepare standards. In order to prepare the working standard solution, the stock solution with the required metal is properly diluted at approximately 1000 grams per liter. To establish calibration curves for metal ions, a working standard of 0 - 40 g/L was used, which was necessary for calculations.

PREPARATION OF MIXED FATTY ACID

Through hydrolysis, fats and oils were broken down into fatty acids. Approximately 2 mL of 1 N alcoholic NaOH and 10 mL of alcohol were added to saponify 1 g of the oil sample. The mixture was then refluxed at a temperature between 60 and 70 °C for duration of two hours, during which TLC observations were conducted. The resulting mixture consisted of both saponified and unsaponified substances. Further dilution of the solution was achieved by adding 30 mL of distilled water. In order to remove the saponified material, multiple washes of diethyl ether were used along with a separating funnel. Unsaponified components were collected from the upper organic (ether) layer. Following evaporation, diethyl ether is collected using a rotary evaporator. Using diluted hydrochloric acid (6N HCl), lower aqueous solutions containing fatty acid salts were acidified. Typically, the fatty acids from this mixture were isolated using diethyl ether. The upper layer of the ether extract, containing various fatty acids, was gathered in a flask that had been dried in an oven after discarding the lower aqueous layer. After evaporating the surplus ether, the mixed fatty acids (MFAs) were purified using double-distilled water and then dehydrated with Na_2SO_4. Thin Layer Chromatography (TLC) was employed to track each step of the process. Using HPLC, the resultant methyl esters were investigated. A coating of silica gel, approximately 0.025 mm thick was applied to a glass plate to make TLC plates. The visible spot observed in the iodine chamber was due to the mobile phase, comprising petroleum ether, diethyl ether, and acetic acid in the ratio of 70:29:1.

HPLC and GC-MS (gas chromatography-mass spectrometry) were employed to convert the fatty acid mixture into esters for the purpose of quantitative analysis. A catalyst of 1% H_2SO_4 was utilized, and the mixed fatty acids (MFAs) along with excess methanol (in a ratio of 1:6) were refluxed at 100°C for 2 hours to produce fatty acid methyl ester (FAME). After confirming that the trans-esterification was complete through TLC analysis, the apparatus was taken apart, and any residual solvent was eliminated by allowing the flask to cool to ambient temperature. Subsequently, 30 ml distilled water was added to the reaction mixture, which was then cool in an ice bath. Fatty acid methyl esters (FAMEs) were extracted from the mixture using diethyl ether. The organic (upper) layer containing the FAMEs was collected into a dry flask, while the aqueous (lower) layer was discarded. The ether solvent was then evaporated, and the residue was dried to obtain anhydrous FAMEs. The resulting FAMEs were stored at a low temperature for subsequent analysis.

ANALYSIS OF FATTY ACID:

Bitter gourd plant oil was analysed using two different methods. In the beginning, hydrolyzing the oil resulted in a range of fatty acids, which were later transformed into methyl esters. We confirmed the production of methyl esters via thin layer chromatography (TLC) and analyzed the methyl esters using high performance liquid chromatography (HPLC). [31]

RESULTS AND DISCUSSION

PHYSICAL PARAMETER DETERMINATION:

The physico-chemical properties of the *Eruca sativa* seed oils, such as their iodine, saponification, peroxide, and acid values, were ascertained using the procedure outlined by AOCS. [32, 33] The values obtained for peroxide, iodine, acid value, and saponification are 4.25, 106, 1.30, and 180, respectively. The specific gravity is 0.88 and the pH is 7.1. 37.50% oil content was found.

Table 1: Physico-Chemical Parameters of *Eruca sativa* Seed oils

S. No.	Characteristics	Value
1	**Oil Content %**	**37.50**
2	**Acid value (mg KOH/g)**	**1.30**
3	**Iodine value (g I_2/100g)**	**106**
4	**Free Fatty acid**	**0.95**
5	**Saponification value (mg KOH/g)**	**180**
6	**Peroxide value (Meq KOH/g)**	**4.25**
7	**Density (g/cm^3)**	**0.98**
8	**Specific Gravity (g/cm^3)**	**0.88**
9	**pH**	**7.1**
10	**Refractive index**	**1.47**
11	**Colour**	**Dark yellow**

FATTY ACID ANALYSIS:

Erucic acid was the most abundant fatty acid in the oil (42.6%), followed by oleic acid (19.4%), linolenic acid (12.4%), palmitic acid (8.4%), and other fatty acids, according to the HPLC of the total lipids (Table 2).

Table 2: Fatty Acid Profile of Oil Extracted from *Eruca sativa* Seeds

S. NO.	Fatty acids	(%) Composition
1	**Erucic acid**	**42.6**
2	**Oleic acid**	**19.4**
3	**Linolenic acid**	**12.4**
4	**Palmitic acid**	**8.4**
5	**Eicosenoic acid**	**6.9**
6	**Linoleic acid**	**6.6**
7	**Stearic acid**	**1.41**
8	**Nervonic acid**	**1.2**
9	**Eicosadienoic acid**	**0.4**
10	**Palmitoleic acid**	**0.1**

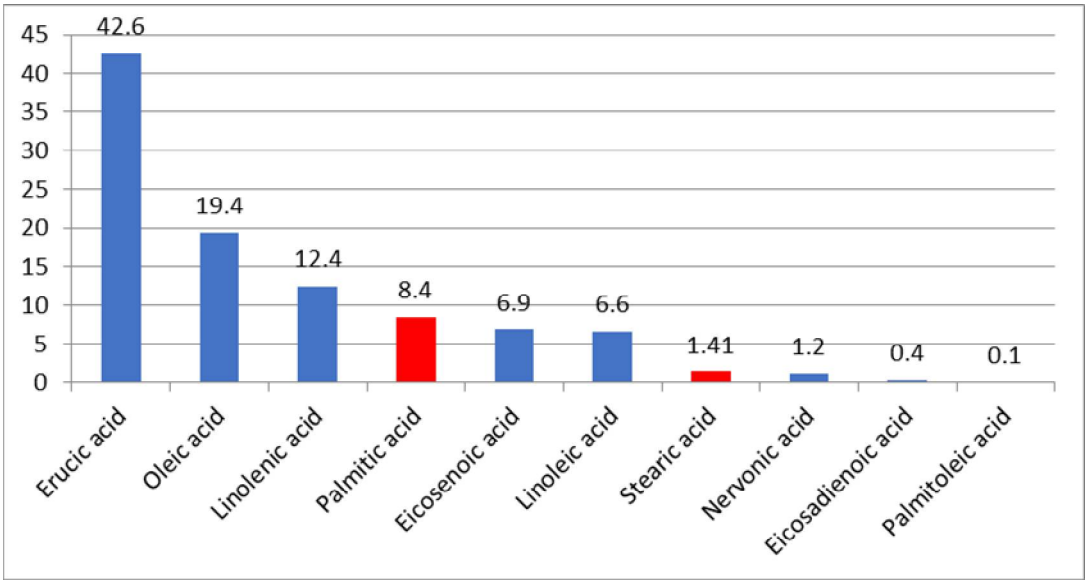

Figure 2: Composition of fatty acids in the oil extracted from *Eruca sativa* seeds

Analysis of Heavy Metal Content:

MP-AES utilizing nitrogen as plasma source gas, was employed to determine zinc, copper, nickel, lead, and chromium concentrations in plant sample. Table 3 shows that Zinc (1.69 ppm), (0.09 ppm), Lead (0.09) and Iron (0.23 ppm) minerals are quite rich in *Eruca sativa* seeds oil whereas Chromium (0.01), Cobalt (0.01), manganese (0.03) and Copper (0.03) minerals are relatively low. Metal substance assurance involved nitrogen fire. MP-AES is employed for the simultaneous multi-element determination of both major and minor components. It utilizes microwave radiation to generate plasma using nitrogen, which

can be extracted from ambient air or supplied through a gas cylinder, thereby eliminating the need for external sourcing of expensive gases such as argon.

Table 3: Concentration of Heavy Metals in *Eruca sativa* Seed Oil

S. No.	Heavy Metal	Concentration (mg/L)
1	Zn	1.69
2	Pb	0.09
3	Ni	0.01
4	Fe	0.23
5	Cr	0.01
6	Cu	0.03
7	Mn	0.03
8	Co	0.01

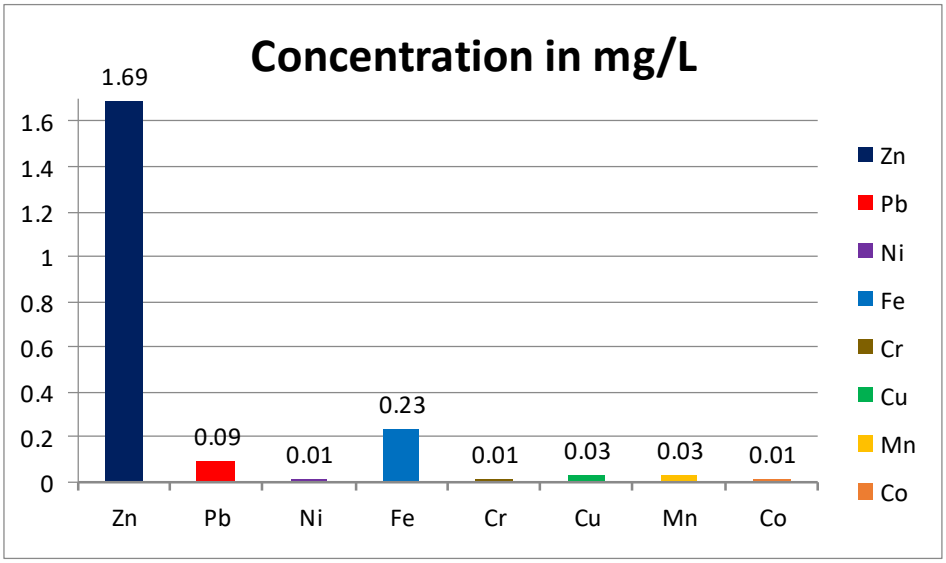

Figure 3: Plot showing comparison between Heavy metal Content

CONCLUSION

This study led us to conclude that the elevated metal contamination levels in the seed oil of *Eruca sativa*, including zinc (1.69 ppm) and iron (0.23 ppm), indicate that this plant is capable of absorbing harmful metals from the soil, as well as through its leaves. Additionally, the high percentage of unsaturated fatty acids in *Eruca sativa* seed oil makes it suitable for industrial and culinary uses (instead of coconut oil). The physicochemical characteristics of *Eruca sativa* oil were examined. The proximate analysis revealed that the oil content of *Eruca sativa* seed oil was 37.50%.

ACKNOWLEDGEMENT

The University Grant Commission (UGC), New Delhi, is acknowledged by Prakash Ram for providing financial support through the award of a research fellowship (NFOBC, UGC ref. no. 202122-RJ07000559). Additionally, we appreciate for giving us MPAES and HPLC data to IIT Roorkee.

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