



ORIGINAL ARTICLE

Evaluation the Growth Potential of Artichoke (*Synara scolymus* L.) and Milk thistle (*Silybum marianum* L.) in Petroleum-contaminated Soil

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KEYWORDS

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ABSTRACT: Petroleum hydrocarbons are one of the most common pollutants groups in the environment and threaten the human, animals and plants health. Phytoremediation is a method for cleaning the contaminated areas. Medicinal plants because of their defense mechanisms able to resist and thwart destructive effect of stressors. Some plants have better resistance, including Artichoke (*Cynara scolymus* L.) and Milk Thistle (*Silybum marianum* L.); from Asteraceae family that has polyphenolic compounds with antioxidant properties and hepatoprotectors. To evaluation the growth potential of Artichoke and Milk Thistle in petroleum-contaminated soil, an experiment in a completely randomized design was done with 6 levels of gas oil and 3 replications in Gorgan University of Agricultural Sciences and Natural Resources. The results showed that, gas oil hydrocarbon had a significant effect at %1 on germination percent of seed and indexes involved in seedling growth including plant height, length, and width, fresh and dry weight of artichoke leaf. In Milk Thistle, gas oil had no significant effect on germination percent. Opposite to that, significant effect at %1 on growth indexes was observed. The maximum germination percent in Artichoke and Milk Thistle seeds was observed in 20 and 10 g/kg gas oil, respectively and the minimum of germination percent was observed in seeds samples that treated with 80 g gas oil per kg soil. Artichoke seedlings were more tolerance than Milk Thistle to the contaminated soil as better growth was observed in this condition. Generally, it seems that these two valuable medicinal plants had relatively resistance to the gas oli pollution and are suggestible to use in oil contaminated soil for cleaning purpose

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INTRODUCTION

Soil pollution to petroleum compounds is a very common environmental problem that seems unavoidable in oil producing countries [1]. The main causes of soil pollution to oil pollutants can be listed as the oil leakage from the pipelines or overflow from its reserve tanks, the accidents of crude oil/product carrying vehicles, and the release of waste and effluents of refineries into the environment [2]. Oil products are common soil contaminants [3], which are poisonous, mutagen and carcinogen. In addition to the deep impact on local ecosystems, they finally find their way into human communities by entering into the food chain and threaten the health of people, plants, animals, rivers, underground waters, and agricultural products [1].

Total Petroleum Hydrocarbons (TPHs) are among the most important stable organic pollutants in the environment that are often emitted into the environment by oil and gas industries. They are a combination of aliphatic, aromatic, heterocyclic and asphaltene hydrocarbons [4]. Gas oil is a variety of petroleum hydrocarbon and a compound with the biological source. It is a naturally occurring substance with very poor water solubility whose presence in the soil causes pollution and toxicity. In natural conditions, the downward movement of gas oil is hindered due to its adsorption by organic matter at the surface of the soil, so the contamination remains at the soil surface and root system of most plant species. Because of this attribute, this pollutant is regarded as an appropriate option for phytoremediation processes [5]. The effects of soils contaminated with petroleum compounds and their byproducts on plants vary with pollutant concentration, the duration of plant exposure, and plant species [6, 7, and 8]. The cultivation of plants in contaminated soils is confronted with some limitations, because they usually encounter a combination of drought stress, nutrient deficiencies and chemical toxicity [9, 10]. The first

symptoms of plant poisoning in oil-contaminated soils are growth inhibition and then, growth reduction. The hydrophobicity of oil compounds changes soil behavior and causes the heterogeneous water distribution in soil. This, in turn, leads to water shortage in soil, resulting in drought and the decrease in water and nutrients availability [11] and the loss of seed germination and the growth of plant organs especially root and shoot. The ultimate consequence is the loss of crop production. The decline in plant growth has been reported in many studies [6, 12, and 13]. In a study on the effect of different concentrations of crude oil on germination and growth of soybean, Ekpo et al. (2012) found that crude oil contamination significantly reduced soybean growth, so that the higher the rates of crude oil was, the more stunted the growth of soybean plants were [14]. The effect of petroleum hydrocarbons on growth, photosynthetic pigments, and carbohydrates of sunflowers showed that sunflowers could survive in soils contaminated with 18 mg kg^{-1} oil pollutant [15]. In a research on the effect of various concentrations of crude oil (0 to 4% v/w) on acacia compounds and its phytoremediation capability, Askari et al. (2012) showed that as crude oil concentration was increased, plant growth was reduced and leaf proline content was increased as a measure of stress resistance [16]. Chaghari et al. (2006) examined the effects of gas oil on germination and growth of some crops such as clover, corn, barley, wheat, alfalfa, and safflower. They reported that seed germination in contaminated soil was largely dependent on plant species. Generally, all studied species showed a retardation phase in the germination of seeds treated with gas oil, so that in the first week, germination percentage was lower than control but it started to increase in the second week [17]. Rangzan and Landi (2006) worked on phytoremediation potential of alfalfa, clover, and barley in removing gas oil from soil, and found that

higher gas oil concentration in soil reduced germination percentage. In general, all three crops showed a good performance refining the contamination, but the highest percentage of contamination removal was related to barley [18].

Soil petroleum contamination can be treated by physical (burning, collecting tools, extracting polluting fumes from the soil, etc.), chemical (extraction by solvents, soil washing, etc.) and biological methods (biological conditioning, phytoremediation, the use of microorganisms, etc.). Chemical and physical methods are used less due to their high costs and harmful environmental consequences. The recent years have witnessed a rise of interest in biological methods [19, 20, 21 and 22]. Among the plants capable of soil remediation, the plants of Gramineae and Fabaceae families have a significant role in remediating contaminated soils due to their extensive root system and nitrogen fixation potential and have been subject to numerous studies [23]. However, studies have been rare on the effect of medicinal herbs on removing petroleum compounds from soil. Due to numerous defense mechanisms and systems, medicinal herbs can resist and neutralize the effects of stress and destructive stressful factors. Some plants e.g. artichokes and marigolds show higher resistance.

Artichoke (*Cynara scolymus* L.) is a medicinal herb from the Asteraceae family. It is native to southern Europe, the Mediterranean area, North Africa, and the Canary Islands. This plant has nutritious flower buds and healing leaves. It leaves contain such important compounds as phenolic compounds, flavonoids, acid compounds and sesquiterpenic lactones (e.g. cynaropicrin). Artichokes have diuretic effects, control blood cholesterol and fat, and is antiemetic and anti-indigestion [24].

Milk thistle (*Silybum marianum* L.) is an annual or biennial crop from the Asteraceae family that grows in Europe, Asia and America. Its seeds contain a variety of flavonoids, including silybin A, silybin B, silydianin, silicristin, and dihydrosi-

lybin. The most important compound of this plant is silymarin that is a combination of flavonoids, flavonolignans, and others with antioxidant, anti-inflammation, and cell glutathione enhancement properties. Silybin is the most effective ingredient of silymarin that is regarded as antioxidant and liver protector. Anticancer effects, lowering blood glucose, reducing fat, controlling nervous system, treating hepatotoxicity, and inhibiting kidney disorders are among the therapeutic effects of this herb [25].

One of the secondary stresses occurring in soils contaminated with oil pollutants is drought. Since these two species can tolerate drought stress, they are expected to be able to grow in these conditions. However, the resistance of plants to stress and stressful factors depends on such parameters such as the concentration of stressful factors like oil pollutants and the duration of exposure to stress. Therefore, this study was aimed to evaluate the potential of these two medicinal herbs in growing in soils contaminated with hydrocarbon compounds and conserving and remediating the soils.

MATERIALS AND METHODS

An experiment was conducted at Gorgan University of Agricultural Sciences and Natural Resources in order to investigate the effect of gas oil (from the group of petroleum compounds) on germination and growth of artichoke and milk thistle. First, the seeds of artichokes and milk thistle were prepared and disinfected. In order to prepare the soil, non-contaminated soil was first prepared and after testing, it was contaminated with 0, 5, 10, 20, 40 and 80 g kg⁻¹ gas oil. It was mixed with soil by hand to ensure its uniform distribution in soil. Then, the seeds were cultivated in pots containing 6 kg of soil contaminated with gas oil and uncontaminated soil (control). The pots were, on average, irrigated every other day as per plant requirement. Plants were cultivated outdoors for a month. Germination percentage was recorded for two

weeks and the following equation was applied to yield the germination percentage:

$$PG = \frac{n}{N} \times 100$$

Where: n represents total number of germinated seeds, and N represents total number of seeds.

Next, vegetative traits were measured with a precision scale including shoot height, leaf length and width, and leaf fresh weight and dry weight. The experiment was based on a Completely Randomized Design with gas oil treatment at six levels in 3 replications. Statistical comparisons were carried

out in SAS Software Package, and the graphs were drawn in MS-Excel Software Package.

RESULTS AND DISCUSSION

According to the results of analysis of variance presented in Table 1, gas oil hydrocarbon compound influenced germination percentage and vegetative traits such as shoot height, leaf length and width, and leaf fresh and dry weight of artichoke significantly at the 1% level. Its impact was insignificant on germination percentage of milk thistle. However, its vegetative traits including plant height, and the length, width, and fresh and dry weight of leaves were significantly affected by the treatment at the 1% level.

Table 1. Analysis of variance of the effect of germination percent and seedling growth of Artichoke and Milk thistle under the gas oil

			Mean squares					
	Sources of changes	df	Germination percent	shoot height	leaf length	leafwidth	leaf fresh weight	leaf dry weight
Artichoke	Treatment	5	782.209 **	5.467 **	3.567 **	0.128 **	0.123 **	0.0009 **
	Error	12	12.5	0.291	0.082	0.017	0.01	0
	CV	-	6.917	6.231	5.456	11.879	12.243	12.094
Milk thistle	Treatment	5	160 ^{n.s}	11.6 **	3.886 **	0.636 **	0.433 **	0.001 **
	Error	12	66.66	0.111	0.102	0.006	0.01	0
	CV	-	11.134	6.896	9.688	7.601	19.773	16.857

** , * , ^{n.s} :Significant in 1%, 5% and no significant

Germination percentage

Figure 1 shows that the highest germination rate (66.7%) of artichoke plants was obtained at 20 g kg⁻¹ contamination level, showing insignificant difference with that of control. The lowest germination percentage was observed in soils contaminated with 80 g kg⁻¹ gas oil. In fact, germination percentage in this soil was 40% lower than that of control. In the case of milk thistle, the highest germination (73.3%) was at contamination level of 10 g kg⁻¹, but it did not differ from that of control significantly. The lowest germination percentage of 66.7% was exhibited by plants grown in soil contaminated with 80gkg⁻¹ gas oil, in which ger-

mination percentage was 6% lower than that of control.

Soil contamination with gas oil hydrocarbon compound suppresses the germination of artichoke and milk thistle seeds. Germination is an important stage of plant growth during which environmental stresses can leave more adverse impacts [26]. Seed germination inhibition and the loss of bud growth in contaminated soils are highly dependent on the concentration of pollutants (petroleum compounds) and plant species [27, 28]. Several studies have focused on the reduced germination and growth of plants in contaminated soils [6, 7 and 28]. associated with the toxicity of petroleum

compounds and their complications. But at certain concentrations, the petroleum compounds in soil can stimulate the growth and biomass production in some species [6] because the stress induced by them can stimulate the construction of some growth regulating agents [29]. This may be the likely reason for the increase in the germination of artichoke and milk thistle seeds at gas oil concentrations of 10 and 20 g kg⁻¹. In general, most studies on the use of petroleum compounds or their derivatives in soil indicate their negative impacts on seed germination [30, 31 and 32]. Adam and Duncan (2002) attributed the loss of germination in soils contaminated with petroleum hydrocarbons to the fact that hydrocarbons in soil, including gas oil, enclose the seeds with an oily layer and hinder their access to water and oxygen, whereby germination is retarded and reduced and embryo dies [28]. This explanation was observed in the germination of ryegrass in soils contaminated with petroleum compounds [33] and corn and red pepper in crude oil contamination [34]. Parvanak et al. (2014) stated that petroleum sludge at different levels had a significant effect on germination percentage of wheatgrass, but its impact was insignificant on flax and alfalfa [35]. In another study, the germination of *Mimosa pilulifera* in contaminated soils did not show significant differences with its germination in unpolluted soils [36]. In our experiment, gas oil had no significant influence on the germination of milk thistle seeds,

which can be related to non-toxicity of the studied rates of the petroleum compound to this species, the type of petroleum compound, and/or the absence of volatile compounds. Also, the high germination speed of some species may be the reason for the ineffectiveness of petroleum compounds on their germination because it shortens the contact time of the seeds with the pollutants and alleviates their toxic effect on them [36]. Zarinkamar et al. (2013) showed that diesel fuel, like other oil compounds, had a negative effect on the growth of *Festuca anundinacea* and that as diesel fuel concentration was increased, germination percentage was decreased during three weeks. The highest quantities of the traits were observed in control (zero level) and the lowest ones in 30000 ppm [37]. In another study, seed germination percentage of *Angropyron desertorum* was lost with the increase in soil petroleum sludge percentage so that the highest percentage (84.55%) was observed at the concentration of 20% which was not significantly different from control which showed 84% germination. The lowest percentage of 51.33% was observed at the concentration of 80% [38]. Askari et al. (2012) observed that acacia germination was decreased significantly at higher crude oil levels. The highest germination of 80% was related to control and the lowest germination of 13.3% was associated with crude oil concentration of 10%. These results are consistent with the results obtained in our experiment [20].

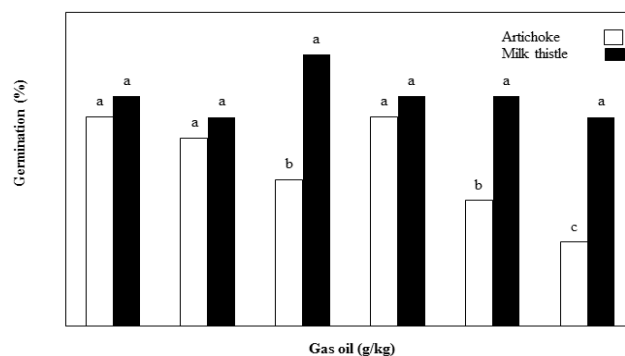


Figure1. Effect of gas oil on germination percent of artichoke and milk thistle

Shoot height

It was found that gas oil had a significant effect on all measured traits of artichoke and milk thistle seedlings including shoot height, leaf length and width, and leaf fresh and dry weight, so that as gas oil dosage was increased, a significant decrease was observed in all parameters. Figure 2 demonstrates that the maximum height of artichoke was 10 cm in control seedlings, which had no significant difference with the seedlings grown at gas oil rates of 5 and 10 g kg⁻¹. Higher concentration of gas oil caused a significant decrease in artichoke height, so that plants grown at 80 g kg⁻¹ gas oil had the lowest height of 6.3 cm. In the case of milk thistle, the tallest seedlings with the height of 7.3 cm were observed in control, but they were not significantly different from that of plants grown at 5 g kg⁻¹ gas oil. The increase in gas oil concentration was significantly related with the loss of shoot height of milk thistle. In fact, plants grown in soils contaminated with 80 g kg⁻¹ gas oil produced the shortest seedlings with the height of 2.7 cm.

The loss of shoot growth of plants in soils contaminated with petroleum compounds and their by-products has been reported by many researchers [6,16 and 39]. The needs that the plants should meet from soil include water, nutrients and oxygen for root respiration and a suitable habitat for root development. Any disruption in them can reduce

or stop the growth of the plant whose impacts are witnessed in shoot [40]. Therefore, the reduction of shoot and plant height in the presence of contaminants in soil, including oil compounds, can be attributed to the lack of soil ventilation which reduces the nutrients, stops growth, and causes plant dwarfness and wilting [41]. Saraeian et al. (2015) stated that the height of wheatgrass shoot in 20% sludge treatment was significantly higher than other treatments, which implies the favorable effect of low dosages of petroleum compounds on the growth of this grass [38]. In another study, petroleum sludge was found to have a significant impact on shoot height of flax and wheatgrass, so that they lost their height as the concentration was increased. Control exhibited the highest height. However, there was no significant difference in wheatgrass between control and 10% petroleum sludge rate [35]. In acacia, plant height was decreased as crude oil concentration was increased, so that control seedlings had the highest height and the shortest seedlings were obtained when the plant growth medium was contaminated with 10% crude oil. The decrease in corn plant height in oil-contaminated soils was also reported by [41], which is consistent with our study.

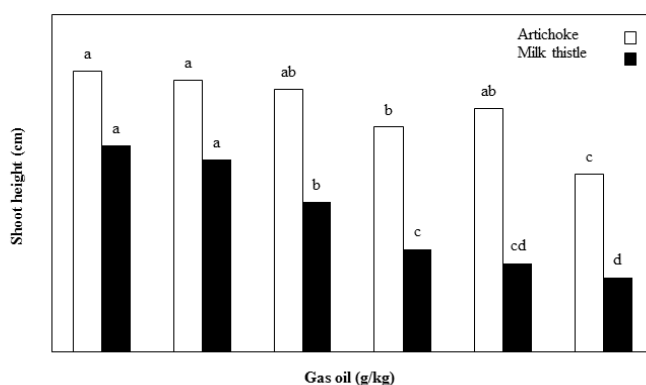


Figure 2. Effect of gas oil on shoot height of artichoke and milk thistle

Leaf length and width

The highest leaf length and width in artichoke seedlings were observed in control (6.27 and 1.30 cm, respectively). They did not exhibit significant differences with those of the seedlings grown in soils contaminated with 5 and 10 g kg⁻¹ gas oil. As gas oil was increased in the substrate, leaf length and width were lost, so that the minimum leaf length and width of 3.53 and 0.773 cm were obtained from gas oil rate of 80 g kg⁻¹, respectively. The examination of leaf length and width of milk thistle seedlings revealed that the maximum length and width were 4.78 and 1.81 cm observed in control leaves, respectively. The leaf length of seedlings grown in 5 g kg⁻¹ gas oil did not differ from control, significantly. With the increase in the concentration of gas oil, a significant decrease happened in leaf length and width, so that the minimum leaf length and width (2,033 and 0.658 cm, respectively) were related to plants grown in gas oil concentration of 80 g kg⁻¹ without any significant differences with gas oil rates of 20 and 40 g kg⁻¹ (Figures 3 and 4).

Soil contamination with petroleum compounds affects the growth and development of leaves adversely too. In stress conditions, it is important to plants to maintain the intra-tissue water [42].But,

the hydrophobic properties of petroleum compounds reduce the moisture content of sediments, so water and food availability to plants is lost [43]and water stress is entailed. The water stress limits leaf development in two ways: reducing leaf size and cell number [27].In fact, the water stress induced by petroleum compounds limits water uptake by roots, which in turn influences the processes like the onset of leaf development, leaf area development and photosynthesis potential negatively [27,42].Acacia leaf area showed a significant decrease when the crude oil was present in the substrate, and the leaf area was decreased further as crude oil concentration was increased. The highest leaf area was observed in control seedlings and the lowest leaf area in seedlings grown in 10% crude oil [20]. Osuagwu et al. (2013) found that the rise in the concentration of crude oil reduced leaf length and area of *Dioscorea bulbifera* L. The maximum leaf length of 11.5 cm was observed in control and the lowest one (6.85 cm) in crude oil rate of 50 mg kg⁻¹ [44]. Kekere et al. (2011) stated that crude oil contamination had a negative effect on leaf number and area of *Vignaun guiculata*. Similarly, we found that gas oil negatively influenced leaf size of artichoke and milk thistle [45].

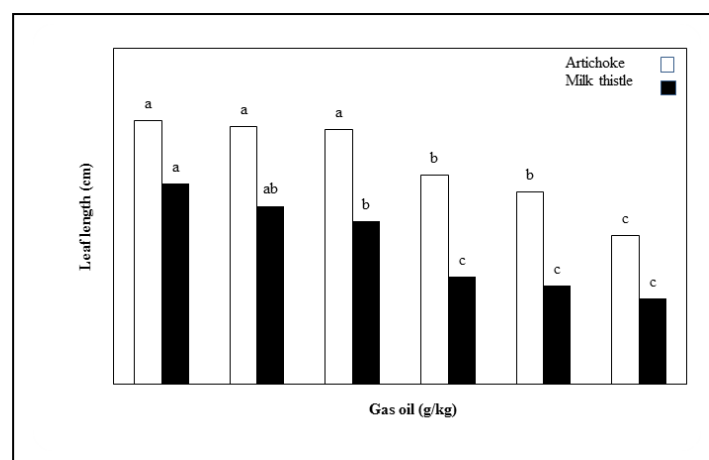


Figure 3. Effect of gas oil on leaf length of artichoke and milk thistle.

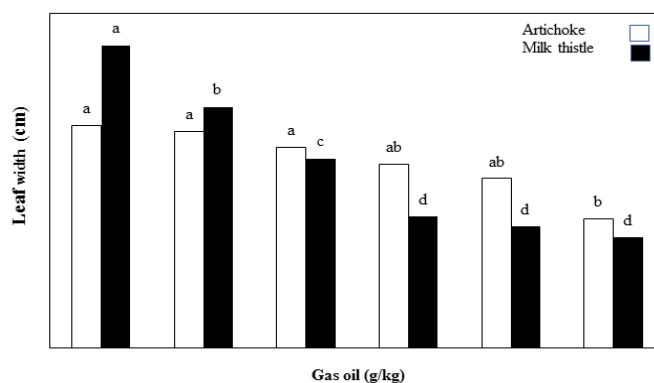


Figure 4. Effect of gas oil on leaf width of artichoke and milk thistle.

Leaf fresh and dry weight

With respect to leaf fresh and dry weight of artichoke seedlings treated with gas oil, it was found that they were decreased as gas oil dosage was increased. The highest leaf fresh weight (1.07 g) was found in control, which was not significantly different from that of the plants treated with 5 and 10 g kg⁻¹ gas oil. The lowest fresh weight of 0.542 g was obtained at gas oil concentration of 80 g kg⁻¹. Leaf dry weight was, also, similar to its fresh weight, so that control plants had the highest leaf dry weight of 0.085 g, which did not have a significant difference from those in plants treated with 5 g kg⁻¹ gas oil. The lowest leaf dry weight (0.039 g) was observed at gas oil rate of 80 g kg⁻¹. The measurement of leaf fresh and dry weight of milk thistle indicated that they were decreased significantly as gas oil concentration was increased. The highest leaf fresh and dry weights (1.128 and 0.069 g, respectively) were observed in control plants. The lowest ones (0.154 and 0.009 g, respectively) were found at gas oil rate of 80 g kg⁻¹ oil, which had no significant difference with the rates of 20 and 40 g kg⁻¹ (Figures 5 and 6).

The hygroscopicity of the soil treated with petroleum hydrocarbons is lost due to hydrophilic prop-

erties of hydrocarbon compounds, disrupting root development [46]. This may, in turn, be brought about by water deficit stress that induces ABA inhibition symptoms in roots [47]. The disruption of root growth and development decreases the absorption of water and food and the growth of all parts of the plant [48]. Then, the reduction of growth, especially in photosynthesizing organs (leaves), entails the loss of biomass and dry matter production. This is the reason for the loss of fresh and dry weight observed in this experiment and other studies. In acacia, a 40-99% loss was found in leaf, stem, shoot, and total fresh and dry weight in the presence of crude oil as compared to control [20].

Chaghari et al. (2006) reported that gas oil had a significant reducing impact on shoot fresh and dry weight of alfalfa and wheat. Corn and safflower plants also lost their shoot fresh weight in the presence of gas oil, but the increase in gas oil concentration did not have a significant effect on plant weight [17]. In our experiment too, the loss of leaf length and width of artichoke and milk thistle plants was followed with the decrease in leaf fresh and dry weight.

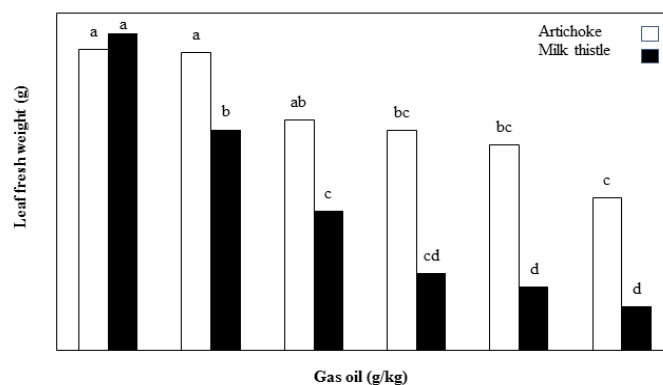


Figure 5. Effect of gas oil on Leaf fresh weight of artichoke and milk thistle.

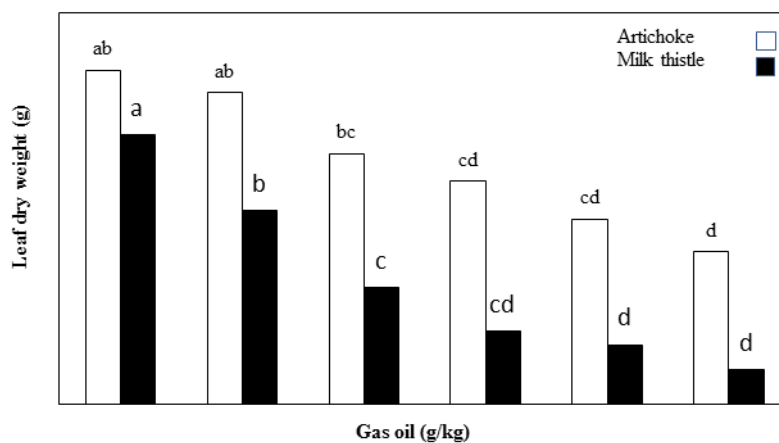


Figure 6. Effect of gas oil on Leaf dry weight of artichoke and milk thistle.

CONCLUSIONS

According to the results, it can be said that soil contamination with petroleum compounds, such as gas oil, has a significant effect on the germination and growth of artichoke and milk thistle. But, these plants could keep their germination and growth, albeit with a decrease but up to contamination rate of 80 g kg^{-1} , implying their resistance to these compounds. In contrary to the artichoke, whose germination was influenced by the concentration of soil contamination with gas oil, the insignificant difference in the germination of milk thistle seeds across different gas oil concentrations indicates better germination of milk thistle seeds in soils contaminated with petroleum residues, particularly gas oil. In contrast to germination, better growth of

artichoke in soils contaminated with gas oil compositions up to $10\text{-}20 \text{ g kg}^{-1}$ and milk thistle in soils contaminated with gas oil concentrations up to $5\text{-}10 \text{ g kg}^{-1}$ indicates better compatibility of artichoke seedlings with adverse environmental conditions. Overall, it can be stated that the planting of these two species in appropriate concentrations can, in addition to their optimal plant growth, purify contaminated soils and create a green cover in polluted areas around refineries. Our previous studies have shown that artichokes are capable of managing the uptake of heavy elements by roots and preventing the accumulation of heavy elements in leaves. In the case of the management of gas oil uptake by roots, which is suggested to be

subjected to further research, the plant can be used for soil purification, green cover creation, and the extraction of plant materials with medicinal value.

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The authors declare that there is no conflict of interests.

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