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Original Research

Assessing the Potential of Roadside Olive as Bioindicator of Metal Pollution in Comparison to Farmland Olive

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Abstract

Heavy metal pollution is a stern global environmental challenge due to its long-term persistence in the environment and bioaccumulating nature. The vehicular emanations having traces of various heavy metals result in contamination of roadside plants and soil. A comparative investigation has been planned between olive varieties Arlik and Gemlik, collected from two sites; roadside and farmland Kallar Kahar. The potential for heavy metal accumulation was explored by comparing the morphological, physiological, biochemical, and antioxidant responses of both varieties from two different sites. Soil and mature olive leaves were sampled, labeled, and brought to the Botany Lab, at the GC Women's University of Faisalabad, for further analysis. The sampling was completely randomized with three replications. The roadside soil showed a high concentration of Pb, Cd, Ni, and Zn and low concentrations of Na and K in comparison to farmland soil. The findings depicted that both Arlik and Gemlik olives experienced high metal stress under roadside conditions. Roadside olive varieties had smaller leaf areas, high fresh and dry weights, fewer amounts of chlorophyll A and B, total chlorophyll and carotenoids, total soluble sugars, proteins, and total free amino acids as compared to farmland olives. However, phenolics, MDA, anthocyanin, and proline antioxidants were greater under roadside stressed conditions. The concentrations of Ni, Pb, Cd, and Zn heavy metals and BAF were higher in both varieties of olive collected from the roadside as compared to farmland. The comparison among varieties showed that Arlik performed far better than Gemlik at both sites.

Keywords: Olive, Bioaccumulation, Kallar Kahar, Roadside, Bioindicator

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Introduction

Soil is the major sink for heavy metals released into the natural environment by anthropogenic events and organic contaminants [1]. In soils, most of the heavy metals are rarely mobile and soluble, with extremely low phytoavailability. Metals frequently appear in residual forms due to effective interaction with biotic or abiotic and organic or inorganic ligands in soils [2, 3]. Heavy metals, such as Zn, Pb, Cu, Cd, Ni, and Fe are emitted via various parts of automobiles. They are observed as the major cause of air pollution worldwide as they emit carbon monoxide, noxious chemicals, volatile organic compounds, the main portion of nitrogen oxides, and some fine particles [4]. Soil contamination by heavy metals from vehicles is a genuine international conservation concern and these metals are released through several processes of road transport. The chief metallic toxins of the edge of the road atmosphere are Zn, Cu, Cd, and Pb, as they are unconfined after battery corrosion, as are metallic parts such as radiators, the outflow of lubricants, the degradation of tires, and fuel burning [5].

In farmlands, sewage sludge, pesticides, and fertilizers are common sources of heavy metal pollution [6]. After heavy metal contamination in the soil, it's extremely tough to rehabilitate the soil ecosystem because of the persistent usage of fertilizers. Farmlands soil has a higher possibility of accumulating Zn, Cu, and Cd [7]. Cadmium intoxication can greatly change plant physiological processes like water relations, photosynthesis, and mineral intake [8]. This metal can simply be engrossed by roots, become part of the food chain, and impose drastic effects on plants, animals, and humans [9]. Long-term Cd exposure causes the root to become necrotic, decomposing, and mucilaginous, which reduces the length of roots and shoots, causes chlorosis, and causes leaf rolling, all of which restrict plant growth [10].

Zn is a fundamental micronutrient and an enzyme unit; several enzymatic responses are initiated by it [11]. In plant roots, the deposit of Zn initiates dangerous injuries; moreover, its high concentration in plant cells is the reason for severe disorders in various plant's physical and biochemical processes, resulting in plant death [12].

Huge amounts of nickel are released into the environment as a result of both natural and human-caused activities such as windblown dust, industrial processes, and volcanic eruptions [13]. Ni is a heavy metal and a crucial micronutrient for the development of plants; therefore, plants turn hazardous when exposed to high Ni concentrations. Therefore, at the cellular level, its high concentration slows down plants' metabolic reactions, generates reactive oxygen species, and causes oxidative stress. An excessive quantity of Ni causes chlorosis and necrosis as well as damages plasma membrane activity [14]. Ni in automobiles discharge generally appears from the wearing of the engine and fluid leakage, and it is a significant metal contaminant whose absorption is quickly increasing in the environment, which harmfully affects the development of plants near roadside areas [15].

Lead (Pb) is the major noxious element for plants near roadside areas, and it is commonly emitted from the exhaust pipes of automobiles resulting from the burning of fuel, where it is added as an anti-knocking agent. In the environment, its concentration is increasing quickly due to the dramatic increment in population and irresponsible human environmental behavior, and large amounts of lead are excluded from the environment through human activities (e.g., burning of fossil fuels, mining), sludge, and industrial management practices [16]. Its uptake by plants is influenced by several soil factors, for example, root surface area, soil texture, pH, and soil structure [17]. High concentrations of Pb in plants affect the reduction of seed germination, reduction of root growth, and inhibition of chlorophyll synthesis [18, 19], as well as affect cell redox status and photosystem II functioning, which then damages its structure [20], and changes membrane permeability, and disrupts the mineral nutrition of the cells [21].

Olea europaea, a well-known species, is extensively distributed in the Mediterranean region, coastal areas of the eastern Mediterranean basin, and the contiguous coastal areas of North Africa, Northern Iran, Western Asia, and Southern Europe [22]. Olive trees prefer loam, sandy loam, and loam soils with moderate moisture around the root zone over wet and heavy-textured loam soils; moreover, taproots grow deeply into the soil. The fibrous roots are shallow; therefore, deep plowing is not valuable for olive groves. The production of olive oil is regarded as an essential asset not only in terms of health and culture but also in terms of the wealth of the farmer from a marginal field, and it is a source of vital fatty acids and antioxidants in human diets [23]. The domesticated olive commercially grown on a large scale presented the enormous potential of this crop in Pakistan [24].

The present comparative study was undertaken to explore the heavy metal accumulation and their possible modulation in the morphology, physiology, and antioxidant attributes of the Arik and Gemlik olive varieties as bioindicators from roadside and farmland sites in Kallar Kahar.

Material and Methods

The current study was designed to comparatively analyze the morpho-physiological and biochemical features as well as examine prospective heavy metal accumulation in olive (*Olea europaea* L.) varieties under roadside and farmland conditions. Two olive varieties were considered for this experiment, i.e., Arlik and Gemlik. The two varieties Arlik and Gemlik, were present along the Kallar Kahar roadside; therefore, the same two were selected from farmland near the village for comparative study. The leaf specimens of selected olive varieties growing in two conditions were collected after taxonomic authentication, adopting a Completely Randomized Design (CRD). Plants of approximately similar size and age were chosen for the current study.

Dirt and soil were gently removed from all olive samples. They were properly stored separately in five plastic self-sealing bags; 20 leaves were present in each bag, dated, labeled, and preserved in an icebox to maintain a low temperature. The leaf specimens of olive varieties were then transported to the Botany Department, GC Women University Faisalabad, where these samples were stored in a refrigerator at 4°C.

The olive varieties chosen for respective assessments were collected from roadside and farmland sites in Kallar Kahar under certain climatic conditions of a semiarid climate, including elevation above sea level of 642m, average temperature of 30.65°C, average annual precipitation of 72.61mm, and relative humidity of 45% during the months of April-May 2023. The morphological, physiological, and biochemical attributes of the collected olive varieties were studied in the Department of Botany, GC Women University Faisalabad, Pakistan.

Soil Studies

The five soil samples were collected from the roadside and farmland sites. For the collection of soil samples, the soil was dug to a depth of 20–25 cm. Soil samples were stored in clean, airtight polyethylene bags and brought to the laboratory for further analysis. Soil samples were then air-dried in the laboratory for 3 days at room temperature. The soil was physically cleaned, homogenized, and sieved through a 2 mm sieve for further analysis, removing visible remnants of leaves and other waste elements.

For the analysis of soil, a soil extract (1:5 *w*/*v*) was prepared. A total of 20 g of the collected soil samples was added to 100 mL of distilled water. This solution was maintained at room temperature in a mechanical shaker for 12 h before being filtered through the Whatman No. 1 filter paper. The filtrate was called soil extract and was used to determine the different parameters, viz., pH, calcium, sodium, and potassium, as well as the concentration of various metal ions including Ni, Cd, Pb, and Zn. The pH was measured by a pH meter, while the calcium, sodium, and potassium content of the soil samples were determined using a Flame Photometer [25]. For the heavy metal estimation, the soil samples were digested using aqua regia following the method described by [26].

Physiological and Biochemical Studies

The fresh weight of the olive varieties under study was stated in grams. The mature leaves, free from herbivore or pathogen damage, were selected from each experimental olive variety. These leaf specimens were placed in pre-weighed paper envelopes and oven-dried at 60°C for 16-24 hours until a constant weight was achieved. The dry mass was determined with an electric balance and expressed in grams [27].

The leaf area of mature leaves of both olive varieties was calculated using the formula proposed by [28].

$$Leaf\ area = \frac{2}{3} \times length\ \times width$$

The leaf pigments Chlorophyll and carotenoids were spectrophotometrically determined by the traditional proposed method [29]. The total soluble sugars [30], total proteins [31], total free amino acids [32], phenolics [33], Anthocyanins [34], Prolines [35], and MDA [36] were analyzed for fresh leaf samples of both olive varieties from both sites.

Metal Accumulation

The dried ground material (0.5 g of root, shoot, and seed) was taken into digestion flasks, and 5 ml of HNO_3 was added to each flask [37]. All the samples were incubated overnight at room temperature. The flasks were placed on a hot plate and heated up to 350°C until fumes were produced, after which heating was continued for another 30 minutes. The digestion flasks were removed from the hot plate, and cooled, and 1 ml of HNO_3 was slowly added to each sample. The flasks were then put back on the hot plate. The above steps were repeated until the digested material became clear and colorless. The volume of the extract was made up to 50 ml with distilled water. The extract was filtered into labeled bottles and used for the determination of different metal ion contents. The biological accumulation factor (BAF) was determined by following the protocol of [38], in which the concentration of heavy metals in plant shoots was divided by the heavy metal concentration in soil.

Statistical Studies

The experiment was Completely Randomized (CRD) with two factors factorial (Olive varieties × Sites) with three replicates. The data recorded for all study parameters was statistically analyzed (ANOVA) using "STATISTICX 8.1" (Analytical Software, Tallahassee, Florida). The statistical significance was tested with LSD ($p \leq 0.05$) represented in tabulated form (Table 1). The graphs were generated using MS EXCEL where bars represent the mean values and error bars represent the standard deviation among means.

Results

At the roadside, Arlik showed an increase in fresh and dry weight (26.18% and 11.11%) in comparison to Gemlik (Fig. 1a, 1b). At the farmland site, Arlik showed high fresh and dry weight (18.76% and 16.39%) as compared to Gemlik. The comparison of the two sites showed that both olive varieties had reduced fresh and dry weights at the roadside than farmland, respectively (Fig. 1a and 1b). The significant leaf area of Arlik (3.82 and 3.37 cm^2) was measured at both farmland and roadside as compared to Gemlik (2.77 and 1.24 cm^2) olive variety (Fig. 1c).

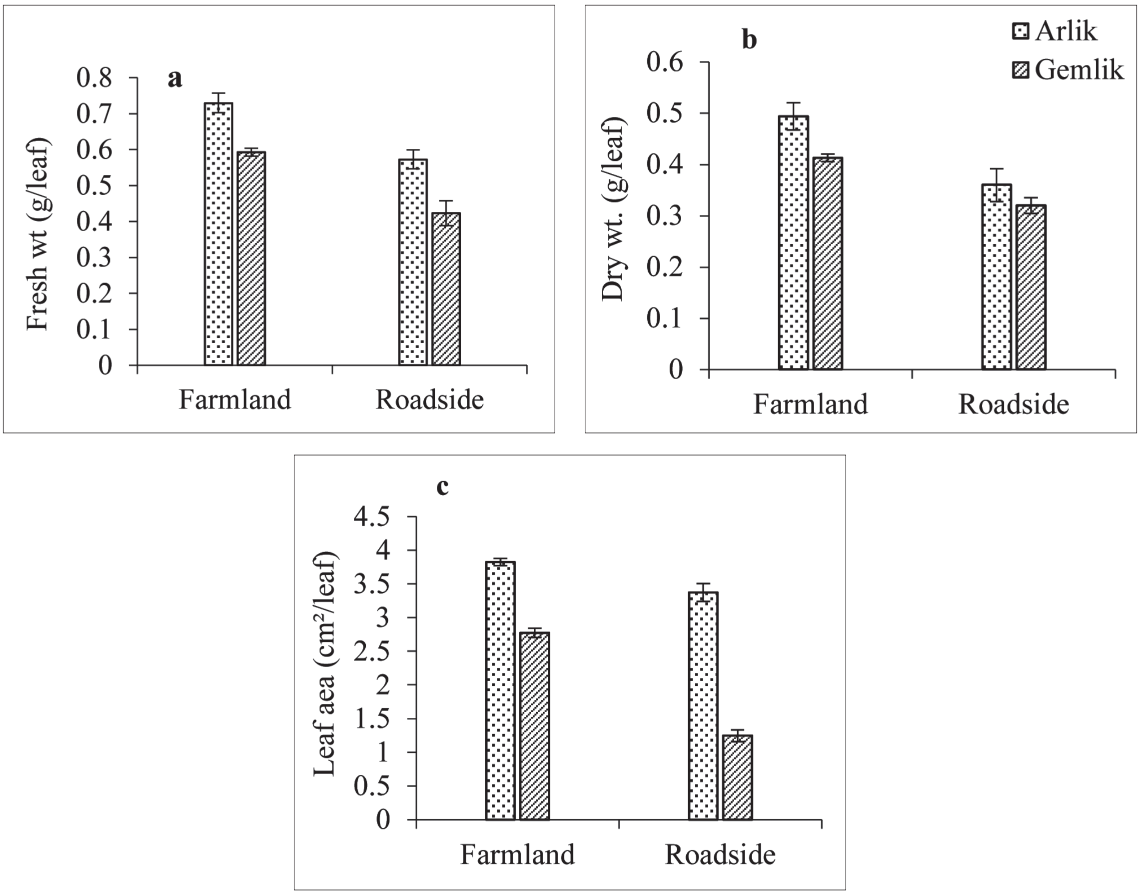

Fig. 1. Fresh weights (a), dry weights (b) and leaf area (c) of Arlik and Gemlik olive varieties collected from Farmland and Roadside, Kallar Kahar

Table 1. ANOVA test of different attributes of Olive varieties at Farmland and Roadside sites

	Sites (S)	**Varieties (V)**	**S×V**	**CV (%)**	**LSD (0.05)**
Fresh weight	0.06***	0.08***	0.0001	6.21	0.048
Dry weight	0.03***	0.01**	0.0009	7.74	0.040
Leaf area	2.94***	7.58***	0.86***	5.62	0.02
Chl a	0.002**	0.0007**	0.00007	10.5	0.009
Chl b	0.0008***	0.000***	0.0003	7.29	0.06
Total Chl	0.57***	0.18***	0.006	2.73	0.09
Carotenoids	0.08***	0.05***	0.0004	6.14	0.04
Anthocyanins	0.18**	0.07*	0.003	14.2	0.13
Phenolics	1.97***	0.14**	0.02	8.23	0.11
Proline	225.5***	130.87***	33.5***	1.15	0.3
MDA	0.38**	0.28*	0.02	1.87	0.21
Sugar	1.01***	6.006***	0.08*	7.36	0.13
Proteins	0.32***	0.36***	0.06*	10.69	0.11
Amino acids	295.02***	146.3***	9.54	1.93	2.71
Ni	31872***	2408.3***	160.3***	4.73	5.01
Cd	1451.9***	3323***	1099.9***	583	4.38
Pb	32970***	1752***	396.75***	2.64	2.9
Zn	1385.4***	389.1***	70.37*	13.06	4.77
			Bioaccumulation factor (BAF)		
Ni	1.46***	0.18***	0.1***	4.8	0.04
Cd	0.55***	0.29***	0.04***	4.87	0.04
Pb	1.48***	0.16***	0.01**	3.32	0.03
Zn	0.03***	0.04***	0.0002	12.6	0.04

Footnote: ***, ** Significant at 1% level of probability, * Significant at 5% level of probability

The varietal difference was significant (Table 1) for chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids at both sites (Fig. 2). The maximum chlorophyll a occurred in Arlik (0.086 mg/g) and Gemlik (0.075 mg/g) at farmland as compared to Arlik (0.062 mg/g) and Gemlik (0.041 mg/g) at the roadside (Fig. 2a). The chlorophyll b was higher at farmland in Arlik (0.067 mg/g), followed by Gemlik (0.054 mg/g) (Fig. 2b). The olive varieties Arlik (0.68 mg/g) and Gemlik (0.55 mg/g) of farmland showed higher contents of carotenoids in comparison to roadside Gemlik (0.04 mg/g) (Fig. 2c). The total chlorophyll (Fig. 2d) in both olive varieties behaved similarly to chlorophyll a and chlorophyll b at both sites.

The soluble phenolics were significant (Table 1) for farmland and roadside for both olive varieties. The concentration of soluble phenolics was enhanced in Arlik (0.94 mg/g) and Gemlik (0.75 mg/g) at farmland, followed by Arlik (0.66 mg/g) and Gemlik (0.55 mg/g) at the roadside (Fig. 3a). At the roadside, Arlik had maximum proline (1.57µmol/g) and Gemlik (1.26 µmol/g) as compared to proline in Arlik (0.67 µmol/g) and Gemlik (0.54 µmol/g) at farmland (Fig. 3b). Arlik variety of roadside had maximum anthocyanins (25.7) and MDA (1.13 nmol/g) as compared to farmland varieties (20.4, 0.87 nmol/g) (Fig. 3c, 3d).

At the roadside, the maximum soluble sugars and total proteins were recorded in Arlik (1.75 and 0.8 mg/g), significantly followed by Gemlik (0.509 and 0.6 mg/g). At the farmland site, Arlik showed high soluble sugars and total proteins (2.507 and 1.286 mg/g) as compared to Gemlik (0.92 and 0.79 mg/g). The comparison of the two sites showed that both olive varieties had more soluble sugars and total proteins on farmland than on the roadside, respectively (Fig. 4a, 4b). The significant total free amino acids of Arlik (114.6 and 105.8 mg/g) were measured at both farmland and roadside as compared to the Gemlik (102.8 and 97.6 mg/g) olive variety (Fig. 4c).

The pH of farmland soil was alkaline (7.45), and roadside soil had an acidic pH (4.98). The comparison of soil samples collected from farmland and roadsides showed great differences in heavy metal concentrations. The essential elements Na, K, and Ca were high at 57, 10, and 189 mg/kg in farmland soil as compared to roadside soil (41 mg Na, 17 mg K, and 93 mg Ca per kg soil, respectively). The roadside exhibited Pb (180 mg/kg soil), Cd (125 mg/kg soil), Ni (121 mg/kg soil), and Zn (139 mg/kg soil) more than farmland soil, in which Pb (63 mg/kg soil), Cd (51 mg/kg soil), Ni (73 mg/kg soil), and Zn (93 mg/kg soil) were present (Fig. 5a).

The comparison of samples collected from farmland and roadside showed great differences in heavy metal concentrations and their biological accumulation factors (BAF). The roadside Arlik exhibited Pb (152.6 mg/kg), Cd (123 mg/kg), Ni (157 mg/kg), and Zn (46.3 mg/kg) more than the farmland Arlik, in which Pb (36.3 mg/kg), Cd (34.1 mg/kg), Ni (30.3 mg/kg), and Zn (20 mg/kg) were present. The heavy metals Ni, Cd, Pb, and Zn in

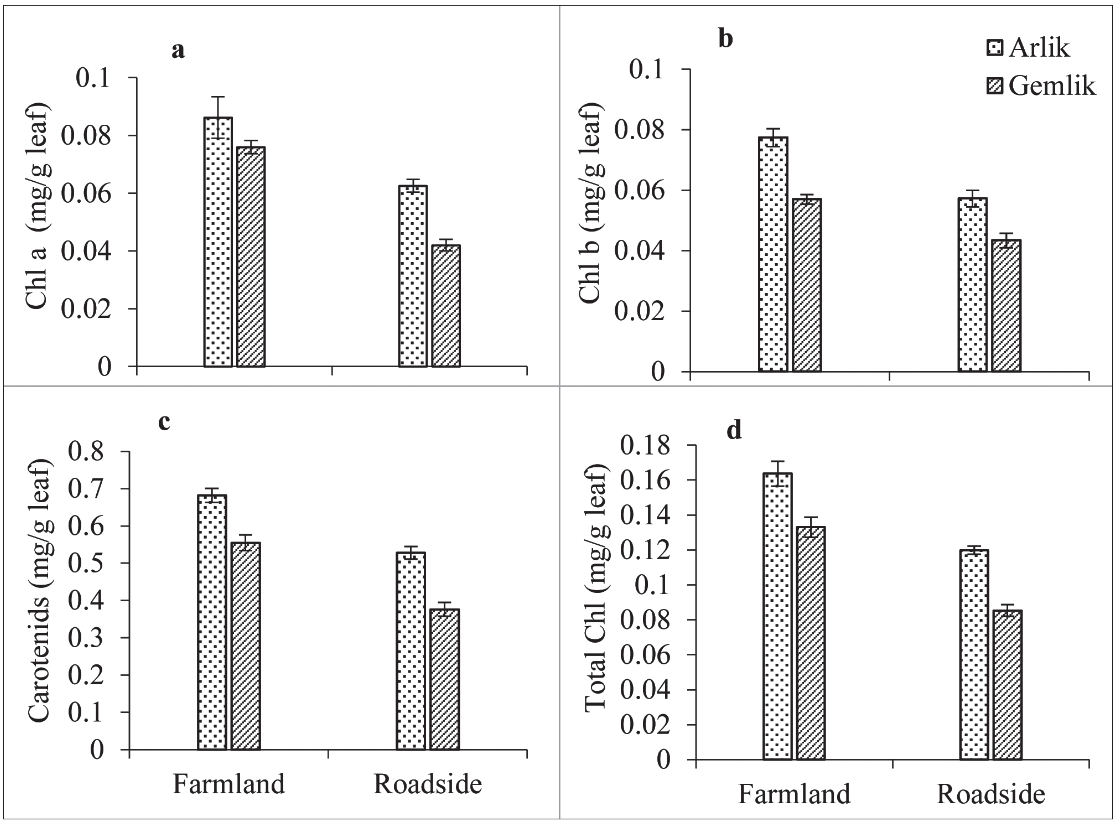

Fig. 2. Chlorophyll *a* (a), chlorophyll *b* (b) carotenoids (c) and total Chlorophyll (d) of Arlik and Gemlik olive varieties collected from Farmland and Roadside, Kallar Kahar

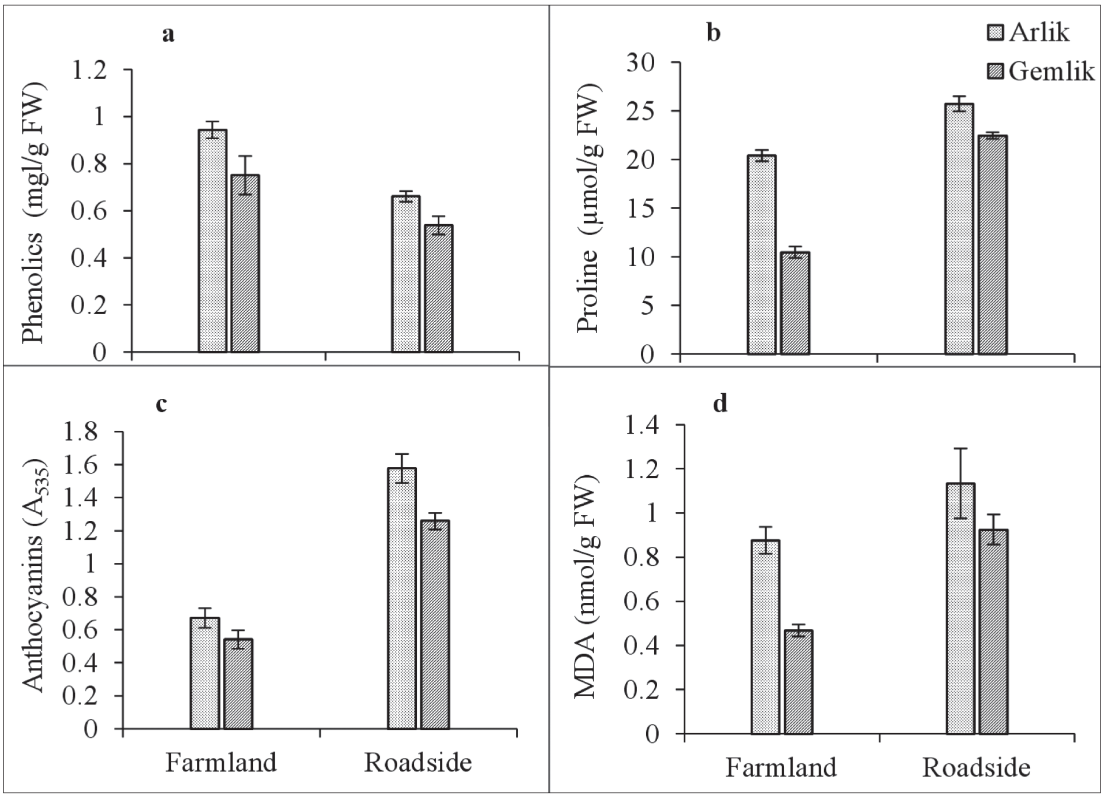

Fig. 3. Soluble Phenolics (a), Proline (b) Anthocyanins (c) and MDA (d) of Arlik and Gemlik olive varieties collected from Farmland and Roadside, Kallar Kahar

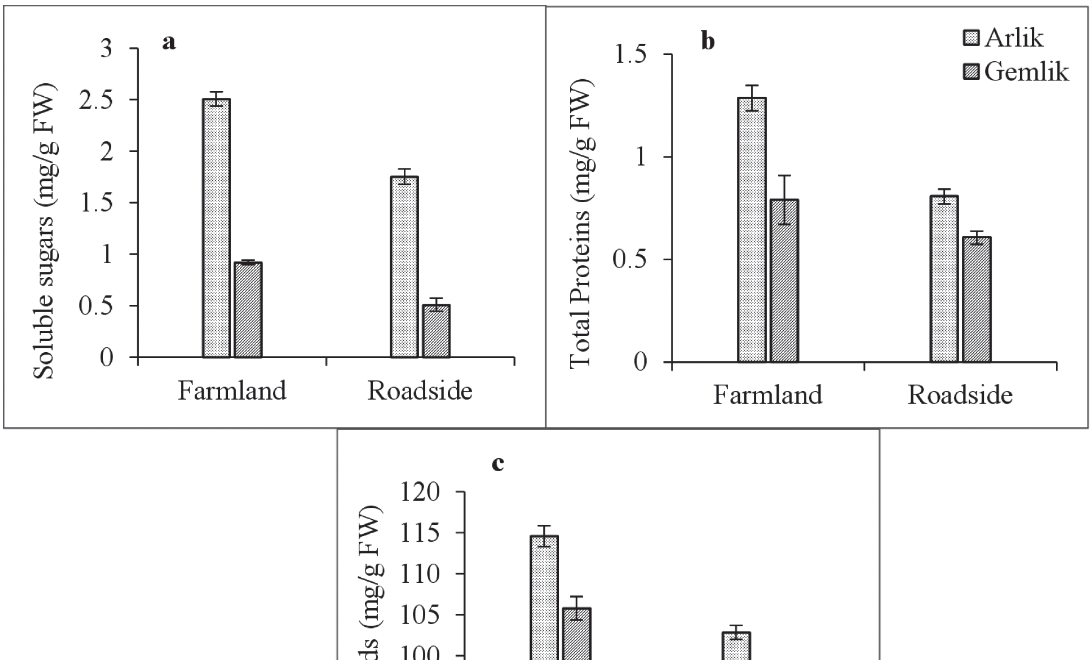

Fig. 4. Soluble sugars (a), Total proteins (b) and Amino acids (c) of Arlik and Gemlik olive varieties collected from Farmland and Roadside, Kallar Kahar

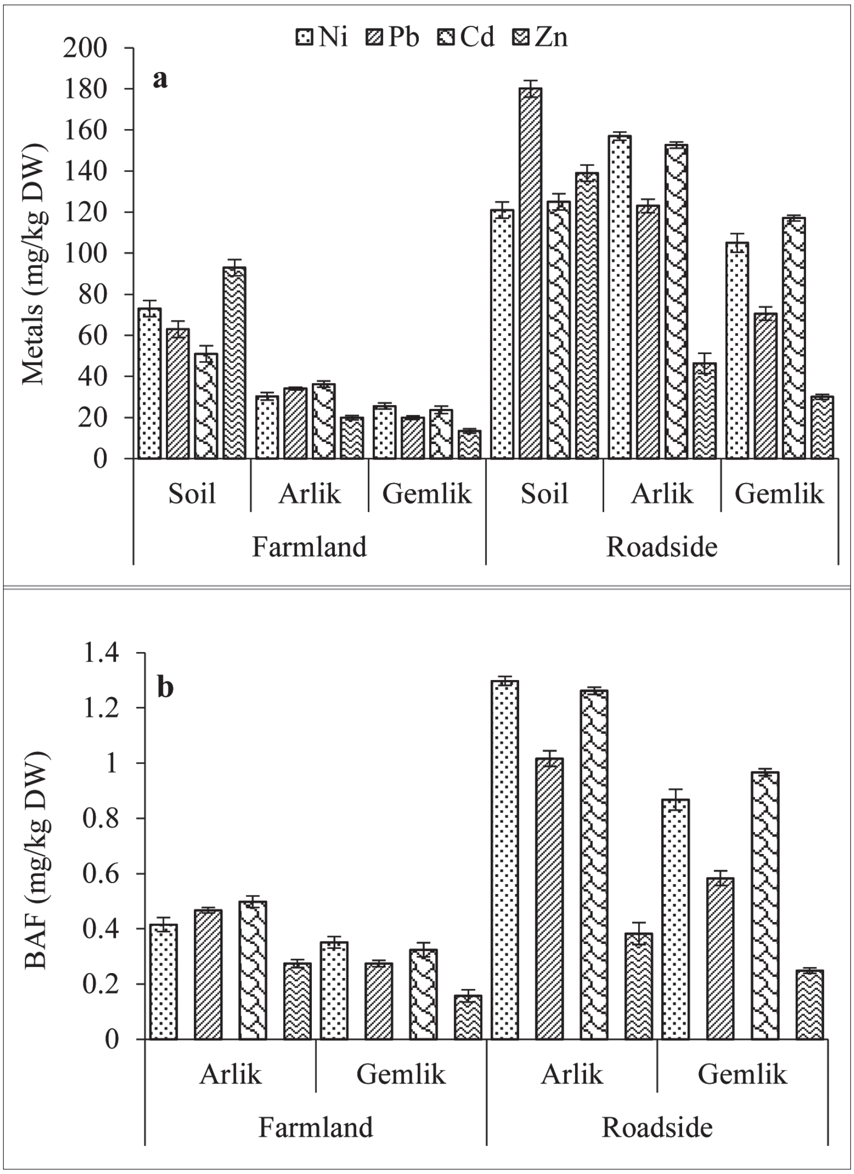

Fig. 5. Metals concentration (a), BAF (b) of soil, Arlik and Gemlik olive varieties collected from Farmland and Roadside, Kallar Kahar

Gemlik were high at 105, 70.5, 117, and 30.1 mg/kg on the roadside as compared to Farmland Gemlik (25.6 mg Ni, 20 mg Cd, 23.6 mg Pb, and 13.5 mg Zn, respectively). Among heavy metals, Ni and Pb BAF (1.29 and 1.26 mg/kg) were greater in Arlik, while maximum BAF in Gemlik was recorded for Ni (0.86 mg/kg) and Pb (0.96 mg/kg) at the roadside as compared to other heavy metals as well as farmland (Fig. 5b).

Discussion

Environmental pollution is a global problem with a large capability to destroy the environment, plants, animals, and human health [39]. Heavy metals are one of the major contaminants that originate from the earth's crust through the weathering of rocks as well as volcanic eruptions during smelting operations, industrial production, and mining [40]. Keeping in view the problem of heavy metal contaminated soil, a comparative investigation had been planned between olive varieties Arlik and Gemlik from roadside and farmland.

The Arlik variety experienced a decline in leaf area, leaf fresh weight, and dry weight under roadside metal contaminated conditions as compared to this variety present in farmland conditions. Similarly, Gemlik present in roadside conditions also behaved in the same manner and showed a reduction in morphological attributes as compared to Gemlik present in farmland conditions (Fig. 2). This declining trend is in line with the morphological attributes of *Nerium oleander* and *Robinia pseudocacia* under metal stress conditions near the major highway of Tehran [41]. As the metals interfere with the nutrient uptake potential of plants, they restrict the uptake of raw materials for growth and development, including K, Ca, and Fe [42].

The influence of heavy metals decreases chlorophyll A, and B, as well as the total chlorophyll and carotenoids [43]. Pb toxicity affects the chlorophyll contents by disturbing photosynthetic machinery in *Cynara scolymus*, which eventually decreases plant growth [44]. The reduction in photosynthetic pigments, including chl a, chl b, total chl, and carotenoids under metal stress has also been reported by [45]. Higher metal content in roadside soil could be the cause of this reduction, which might be the outcome of disrupted chlorophyll biosynthesis as a result of enzyme inactivation including, δ-aminolevulinic acid dehydratase and protochlorophyllide reductase, which are the key enzymes for the biosynthesis of chlorophyll [46]. Moreover, metals (Cd, Pb, Cu, and Ni) could also be the cause of straight oxidative stress by replacing the central Mg atom in chlorophyll molecules, resulting in degraded chlorophyll structure and content. Proteins like chlorophyll a/b binding proteins and photosystem II subunits, which are responsible for chlorophyll biosynthesis and also play a major role in the photosynthetic process, also got affected by heavy metal stress, resulting in reduced chlorophyll content [47]. Carotenoids assist the plants by quenching the oxidizing agents [48]. However, the protective properties of carotenoids are modified by stress conditions, causing pigment degradation and cellular disturbance [49]. The pollutants originated from various vehicular activities and caused a mass reduction in photosynthetic pigments in plants collected from roadside areas of Karachi [50].

During the current investigation, the Arlik variety experienced a reduction in all the biochemical parameters under investigation, including total soluble sugar, protein, and total free amino acids, under roadside metal contaminated conditions as compared to this variety present in farmland conditions. Similarly, roadside polluted conditions suppressed the biochemical parameters in Gemlik in comparison to farmland (Fig. 4). In roadside samples of plants, high metal content might be the cause of this reduction in biochemical attributes. The reduction in protein content may be the result of enhanced degradation or inhibition of formation by heavy metal stress [51]. It may also be argued that enhanced activity of proteolytic enzymes, i.e., protease, may be the reason for this reduction, as well as elevated protein hydrolysis in response to the catalytic activity of metals accumulated in plants [52].

The content of amino acids in both varieties of olive sampled from the roadside with reference to farmland plants is attributed to metal stress. The outcomes of this study are in conformity with the previous findings of many scientists, who stated that an increase in amino acid content is the result of the noxiousness of metals. The increase in total free amino acids is due to the disruption of cell membranes and the breakdown of protein structures and enzymes under heavy metal stress. The accumulation of various metabolites and free amino acids rescues the plants from heavy metal stress by binding with metallic particles. The activation of the plant's defense system in terms of metabolites under stressful conditions protects the plants from the damaging effects of metal stress [53]. Total soluble sugar content decreased drastically under roadside conditions in Arlik and Gemlik in comparison to their presence in farmland. Sugars are recognized as compulsory metabolites as the initial complex organic molecule formed because of the photosynthetic activity of plants as well as the major energy source through respiration. They also have an important role against various infections and stresses, including metal stress. Heavy metal stress reduces the amount of total soluble sugars, and this may be due to damage to the photosynthetic machinery and a high intake of energy due to oxidative stress and noxiousness responses [54]. Metals Pb, Cd, Cu, and Zn boost the antioxidants MDA, anthocyanins, proline, and phenolic contents of *Plantago lanceolata* and *Cardaminopsis arenosa* [55]. Heavy metal contamination along busy highways is the reason for the high antioxidant content of maple leaves [56]. In the present study, there is a positive correlation between heavy metal stress and the content of antioxidants in plants sampled and studied under roadside conditions.

Calotropis procera gathered higher Pb content in above-ground parts along highways with heavy traffic [57]. Metal bioaccumulation differs significantly among different plant species growing in the same area and even within species along different polluted roadsides [58]. The combustion of oil exhaust fumes and the wearing and tearing of moving engine parts mainly release Zn, Cd, Pb, and Ni in the roadside environment [59]. The translocation and bioaccumulation factors of polluted sites are a result of the high content of various heavy metals influenced by various soil factors. It can be argued that the transfer of metals from soil solutions to plants is a natural phenomenon that is dependent on different factors such as nutrients, soil structure, ion exchange capacity, clay content, organic matter content, pH, and most importantly, the concentration of metals in soil. They are taken up by various concentrations, so the high value of various concentration factors such as BCF, BAF, and TF indicated the enhanced transfer and accumulation of metals from rooting medium to plants [60, 61].

Conclusions

The concentrations of Ni, Pb, Cd, and Zn heavy metals and BAF were higher in both varieties of olives collected from the roadside as compared to farmland. The comparison among varieties showed that Arlik performed far better than Gemlik at both sites. Metal accumulation (BAF) damaged the chlorophyll contents and membrane integrity, which was protected by an increase in the amount of MDA, anthocyanins, phenolics, and proline. Further studies at the genetic and omics levels will help in the selection of heavy metal-tolerant olive varieties and their cultivation at the commercial level in Pakistan.

Ethical Issues

There are no ethical issues associated with this publication.

Author Contributions

All authors have contributed equally and there is no conflict of interest.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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