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INDICATIVE AND REMEDIATIVE PROPERTIES OF *VICIA FABA* L.

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Abstract

The study examined the impact of soil contamination with crude oil on the developmental stability of the plant *Vicia faba* L. - broad bean as well as the amount of heavy metals in soil and plant leaves. The experiment was conducted under laboratory conditions with the influence on soil various amounts of oil addition. Deviations from the stability of plant development were studied based on the amplitude of fluctuating asymmetry indicators of bilaterally symmetrical broad bean leaves. Research has shown that the level of soil contamination with oil is directly proportional to the values of fluctuating asymmetry indicators. The heavy metal content (Mn, Fe, Zn, Cu, Co, Pb, Ni, Cr, Sr and V) in the soil and leaf samples was determined by X-ray fluorescence spectroscopy. The main advantages of XRF spectroscopy technique is non-destructive sample preparation - the absence of the stage of destruction of the soil and vegetal samples by chemical reagents or high temperature. The capacity of *V. faba* plant to extract and accumulate heavy metals in leaves was determined by using the bioaccumulation coefficient. It was found that *V. faba* leaves accumulate Zn and Cu. The results of this study may be useful for assessing the feasibility of using broad bean as an ecological indicator and remediator of oil-related soil pollution.

Keywords: oil contamination; heavy metals; *Vicia faba* L.; bioindication; remediation.

1. Introduction

In the Caspian Sea region, Azerbaijan is a significant producer of crude oil and a significant exporter of crude oil and natural gas. The Absheron peninsula is the main oil-producing area and the most economically and socially developed region in Azerbaijan. Hence, the contamination of landscapes with oil and oil-derived products is one of the major issues that have developed here since the start of the exploitation of oil resources [1, 2, 3]. The current level of soils' oil pollution makes research in the field of assessment of the pollution degree and remediation of soils quite relevant.

Oil contamination harms soil biocenosis, changes soil chemical composition, structure, physical properties, and reduces soil fertility and agricultural value. Oil spills transform soils into man-made deserts with no

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biological activity. Oil-contaminated soils are unusable for agriculture or recreation, besides contaminated soils create a pollution risk of surface and ground waters [4].

Consequently, soil contamination by oil has a negative impact on plant growth and development, due to the release of toxic by-products [5, 6]. Particularly, among other pollutants, crude oil contains heavy metals as well. Although some of the heavy metals such as Cu, Fe, Zn, Mg, Mn, Cr and others are essential in low concentrations for cellular metabolism, but their accumulation in higher concentrations can have toxic and carcinogenic effects [19, 20]. Heavy metals have a toxic effect on soil organisms, affecting the main microbial processes and reducing the abundance and activity of soil microorganisms. Even low concentrations of heavy metals can inhibit plant metabolism. The uptake of heavy metals by plants and their subsequent accumulation along the food chain is a potential risk to animal and human health [7].

Therefore, it is important to develop quick and at the same time effective methods for determining the level of soil pollution by heavy metals. Living things used to measure environmental pollution levels and evaluate environmental dangers are called "bioindicators" [11]. According to the literature, plants can be used as reliable bioindicators for assessing soil pollution [8, 9, 10].

Under stressful conditions, changes occurring in the living organism's body at the microscopic level of organization can be evaluated at the macroscopic level by indicators of fluctuating asymmetry (FA) of bilaterally symmetric morphological traits [12, 13]. FA is small, random deviations from perfect symmetry and characterizes developmental instability of an organism [13, 17]. A significant number of both environmental and genetic factors can induce change of FA levels of bilateral traits [15]. Studies have shown that the increase in the content of heavy metals in the soil leads to a violation of the stability of plant development, which is reflected in the FA indicators of their leaves [10, 14, 15, 16]. So, FA of plants leaves can be used as an indicator of assessment the degree of soil pollution by heavy metals.

Along with assessing the level of soil contamination by heavy metals, it is critical to clean-up of heavy metals from contaminated soil. Phytoremediation is the most generally recommended green technology for it. İt is a soil-friendly and economically effective alternative to engineering techniques that are usually more destructive and expensive [18]. The effectiveness of phytoremediation depends on the proper selection of plant species with high accumulative capacity.

Taking into account all the above, this study examines both bioindicative and phytoremediative prospects of the plant *Vicia faba* L. in the conditions of crude oil-contaminated soils. The study aimed to determine the effect of oil pollution on the content of heavy metals in the soil, on the stability of the development of morphological traits of V. *faba* leaves, and on the accumulative properties of this plant.

2. Materials and Methods

The object of the study was the broad bean - *Vicia faba* L. Broad bean belongs to the genus *Vicia* L. of the Legume family (*Fabaceae*) [21]. It is one of the oldest legumes in the world and is used for both human food and animal feed due to its 30% protein content [22]. Nowadays, broad beans are a major crop in many countries including Azerbaijan. *V. faba* has excellent potential to provide the food security of Azerbaijan due to its nutritional value and scale of use.

The experiments were carried out under laboratory conditions.

Soil for the experiment was taken at a depth of 0–30 cm, from the village of Goradil (40°32'49.9"N 49°49'31.4"E). According to the Ministry of Ecology and Natural Resources of Azerbaijan, this village is situated in an area of the Absheron Peninsula (Republic of Azerbaijan) that is relatively ecologically clean and far from traffic, dust, noise, and other kinds of anthropogenic pollution. In the Goradil area of Absheron peninsula widespread soil type is gray-brown soil [33]. Soil samples were placed in a plastic pot, per 100 g of soil in each. To investigate how varying oil concentrations affected the levels of heavy metals in soil and plant leaves, crude oil was added by pipette to each pot with soil sample in the amounts of 1, 10, and 30 ml. The oil added to the pots was mixed thoroughly with the soil. The oil-free soil sample was used as a control. For experiments, ten samples for each dosage of oil were prepared. One seed of *V. faba* was planted in each pot.

The soil samples contaminated with 1, 10, and 30 ml of crude oil conventionally designated respectively as follows: S-1; S-10; S-30. And, the leave samples of plants cultivated in pots filled with soil contaminated with 1, 10, and 30 ml of crude oil symbolized respectively as L-1; L-10; L-30.

To analyze the impact of soil oil pollution on the developmental stability of the plant *V. faba*, we investigated the FA of its leaves [23, 24]. Measurements were made on completely formed leaves of 45-day-old plants. The biometric traits on the left and right halves of every leaf were measured to an accuracy of 0.1 mm. The bilateral difference (BD), fluctuating asymmetry coefficient (FAC) and dispersion (D) were calculated according to known formulas [25, 26] by the specially developed statistical software "STATISTIK 6" [27].

The concentration of heavy metals (Mn, Fe, Zn, Cu, Co, Pb, Ni, Cr, Sr and V) in the soil and leaf samples were determined by rapid and effective X-ray fluorescence (XRF) spectroscopy method on the device "Rigaku NEX QC+" (Applied Rigaku Technologies, Japan) [32]. The samples were irradiated using an X-ray tube operated at 50 kV (Ti to U). Helium was used as carrier gas. The leaf samples were washed in distilled water to remove any dust. To remove moisture, the leaf and soil samples were dried in a laboratory oven at 65 °C and 40°C respectively, until constant weight was obtained. Dried leaf and soil samples were ground to 1 mm size in a ball mill "Herzog" (HERZOG Maschinenfabrik, Germany). 1 g of powdered samples pressed to pellets by using compact pulverizing mill and pellet press "Herzog HP-MP" (HERZOG Maschinenfabrik, Germany) on a substrate of boric acid [30, 31].

The bioaccumulation coefficient (BAC) was used for assessing the plant's capacity to accumulate heavy metals from soil [28].

3. Results and discussion

The results of quantitative determination of heavy metals in soil and leaf samples are presented in Table 1. The obtained results show that in the control soil the content of heavy metals does not exceed their maximum permissible limit values [29].

Table 1. Selected heavy metal contents in the samples of soil and *V. faba* leaves (mg/kg).

Heavy metall	control soil	S-1	S-10	S-30	control leaves	L-1	L-10	L-30
Cr100	90	130	140	140	20	20	20	20
Mn2000	650	1050	1120	1160	60	80	80	90
Fe50000	10410	45390	45600	45640	1520	2300	2640	2760
Co50	10	40	40	50	-	-	-	-
V130	50	80	100	110	20	40	40	40
Ni50	40	90	150	150	10	30	40	40
Cu100	80	120	170	210	100	120	130	130
Zn300	70	110	130	170	60	80	90	110
Sr500	310	350	350	350	10	10	10	10
Pb100	30	70	70	70	-	-	-	-

The concentrations of Fe range in the soil samples from 10410 to 45640 mg/kg and in the leaf samples from 1520 to 2760 (Fig. 1). Among soil and leaf samples, the lowest Fe content is at the control samples. As the concentration of Fe in soil samples increases by increasing the amount of crude oil contamination, Fe content in leaves increases accordingly. The concentration of Fe in all soil samples is within its maximum permissible limit value (50000 mg/kg) [29]. But, in all leaf samples the Fe content is higher than its suggested maximum concentration value (750 mg/kg) [34].

Mn content varies within (650-1160) mg/kg in the soil samples and within (60-90) mg/kg in the leaf samples (Fig. 1). The highest Mn content is in the soil and leaf samples contaminated with 30 ml of crude oil and the lowest Mn concentration is in the control soil and leaf samples. Mn concentration does not exceed the maximum permissible limit in all soil samples (2000 mg/kg) [29] and suggested maximum concentration value in all leaf samples (300 mg/kg) [34].

The low Sr content (310 mg/kg) is in the control soil sample (Fig. 1). In soil samples contaminated with different oil concentrations Sr content does not change and is equal to 350 mg/kg. Sr concentration does not exceed the maximum permissible limit in soil samples (500 mg/kg) [29]. All leaf samples contain the same amount of Sr (10 mg/kg).

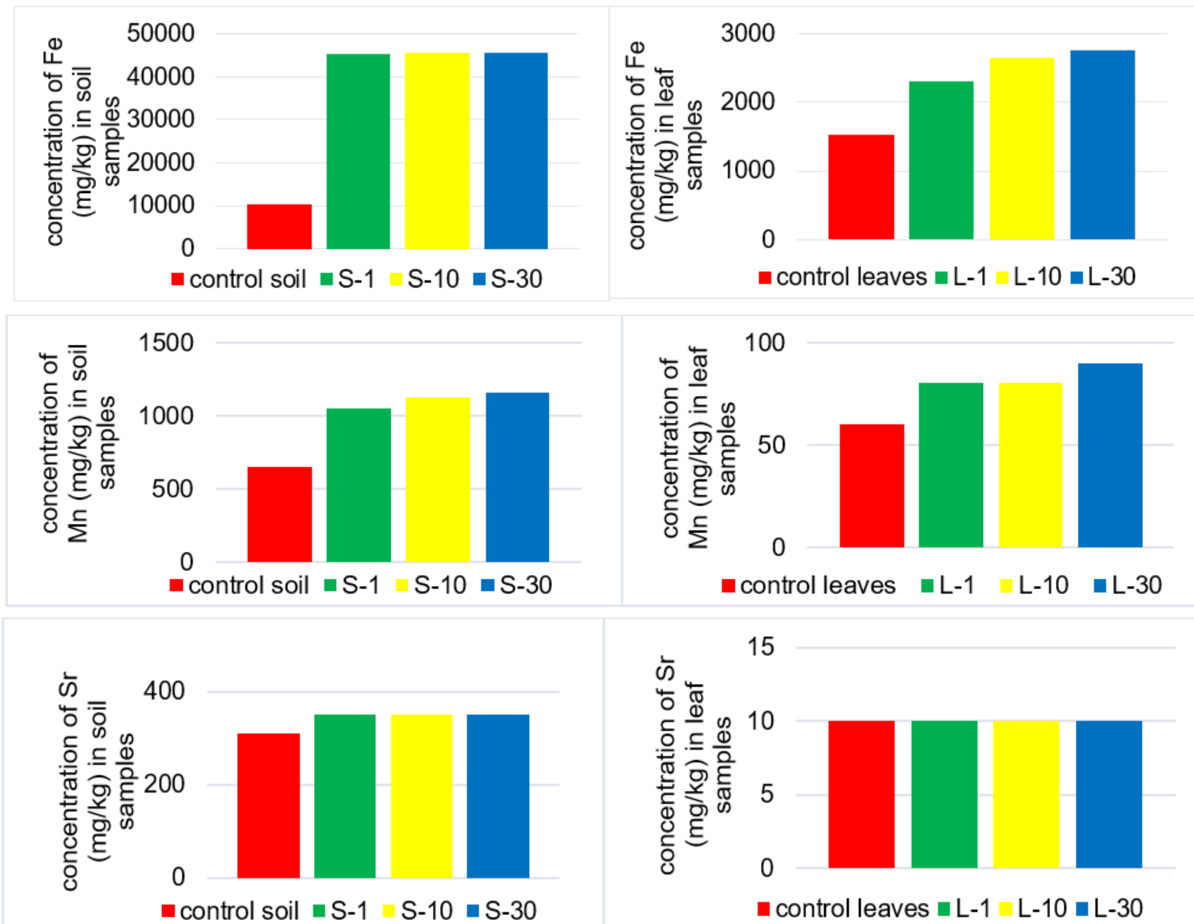

Fig.1. Graphical representation of the concentration of iron (Fe), manganese (Mn) and strontium (Sr) in soil and leaf samples.

Increasing the degree of crude oil pollution leads to a significant increase in Cu concentration in soil samples (80-210 mg/kg) (Fig. 2). While Cu content in leaf samples of plants grown on crude oil-contaminated soils increases not so much (100-130 mg/kg). The lowest Cu content is in the control soil sample (80mg/kg) and control leave sample (100 mg/kg). The highest Cu content is in the soil sample contaminated with 30 ml of crude oil (210 mg/kg). The leaf samples of plants grown on soil samples contaminated with 10 and 30 ml of crude oil have the highest Cu content (130 mg/kg). The values of Cu content in the soil samples contaminated with crude oil are above the maximum permissible limit in soil (100 mg/kg) [29], while all leaf samples remain below the suggested maximum concentration in plant leaves (150 mg/kg) [34].

The Cr content in the soil samples ranges between (90-140) mg/kg (Fig. 2). The highest concentration of Cr recorded in soil samples contaminated with 10 and 30 ml of crude oil (140 mg/kg), while the lowest concentration is in the control soil sample (90 mg/kg). Despite the variation in Cr content in the soil samples, the Cr content in the leaf samples does not change and remains constant (20 mg/kg). In all soil samples contaminated with crude oil, Cr concentration exceeds the maximum permissible limit (100 mg/kg) [29]. Although, the Cu content in leaf samples does not exceed the phytotoxic value (30 mg/kg) [35].

Zn concentration ranges from 70 to 170 mg/kg in soil samples and from 60 to 110 mg/kg in leaf samples (Fig. 2). The lowest Zn concentration recorded in the control soil sample (70 mg/kg) and control leaf sample (60 mg/kg). The highest Zn content is in the soil sample contaminated with 30 ml of crude oil (170 mg/kg) and leaf sample of plants grown on this soil (110 mg/kg). It was observed that as the Zn content increased in soil samples, it also increased in leaf samples. Zn content in all soil samples is below the maximum permissible limit (300 mg/kg) [29]. In addition, Zn content in all leaf samples is within the suggested maximum concentration in plant leaves (300 mg/kg) [34].

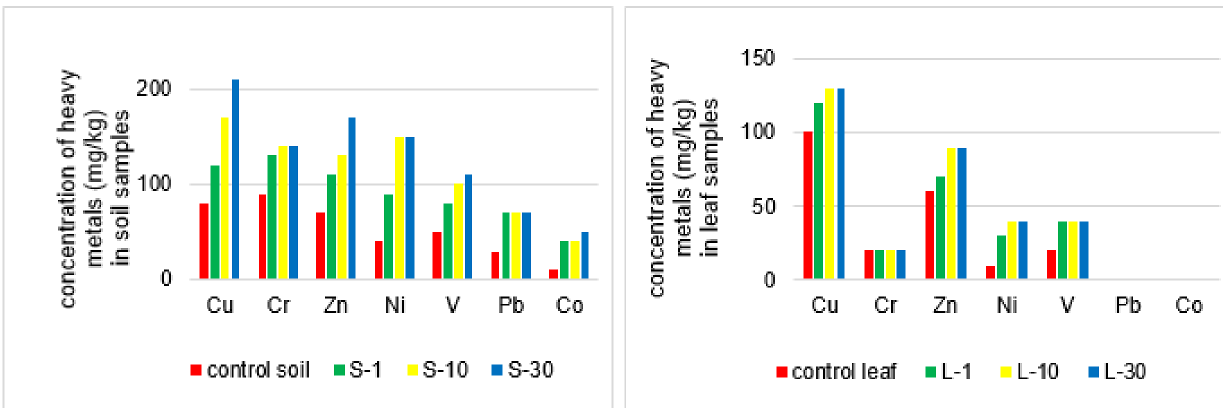

Fig. 2. Graphical representation of the concentration of heavy metals: copper (Cu), chromium (Cr), zinc (Zn), nickel (Ni), vanadium (V), lead (Pb), cobalt (Co) and in soil and leaf samples.

Ni concentration in soil samples varies between (40-150) mg/kg and in leaf samples between (10-40) mg/kg (Fig. 2). The control soil sample (40 mg/kg) and control leaf sample (10 mg/kg) have the lowest Ni content. The highest Ni concentration is in the soil samples contaminated with 10 and 30 ml of crude oil (150 mg/kg) and in leaf samples of plants grown on these soil samples (40 mg/kg). It is important to note that in all soil samples except the control sample, the Ni content exceeds the maximum permissible limit of 50 mg/kg [29]. Similarly, all leaf samples have Ni content above the suggested maximum concentration in plant leaves of 3 mg/kg [34]. Additionally, the Ni content in all leaf samples falls within the range of phytotoxic values of 10-100 mg/kg [35].

The lowest V concentration is in the control soil sample (50 mg/kg) and the control leaf sample (20 mg/kg) (Fig. 2). As crude oil concentration increases in soil samples, V content also increases and varies between (80-110) mg/kg. However, despite the increase in V content in soil samples, it remains constant at 40 mg/kg in leaf samples. The V content in all soil samples is within the maximum permissible limit of 130 mg/kg [29]. Nevertheless, the V content in all leaf samples exceeds the suggested maximum concentration in plant leaves of 2 mg/kg [34] and the range of phytotoxic values of 5-10 mg/kg [35].

The control soil sample contains the lowest concentration of Pb, which is 30 mg/kg (Fig. 2). In all soil samples contaminated with different concentrations of crude oil, the Pb content remains constant and is 70 mg/kg. The Pb content in all soil samples is below the maximum permissible limit of 100 mg/kg [29]. Although Pb was found in all soil samples, Pb was not detected in leaf samples.

The concentration of Co in soil samples ranges from 10 to 50 mg/kg (Fig. 2). The control soil has the lowest concentration of Co of 10 mg/kg, whereas the soil sample contaminated with 30 ml of crude oil has the highest concentration of 50 mg/kg. It is important to note that the Co content in all soil samples is below the maximum permissible limit of 50 mg/kg [29]. Although Co was present in all soil samples, it was not detected in any of the leaf samples.

The results of heavy metal concentration analysis in soil samples showed a specific order for each sample. If we arrange heavy metals in descending order of their concentrations in the studied soil samples, we can obtain the following order for the control soil: Fe > Mn > Sr > Cr > Cu > Zn > V > Ni > Pb > Co. For soil samples contaminated with 1ml of crude oil, the order is Fe > Mn > Sr > Cr > Cu > Zn > Ni > V > Pb > Co. For soil samples contaminated with 10 ml of crude oil, the order is Fe > Mn > Sr > Cu > Ni > Cr > Zn > V > Pb > Co. Lastly, for soil samples contaminated with 30 ml of crude oil, the order is Fe > Mn > Sr > Cu > Zn > Ni > Cr > V > Pb > Co. In summary, the concentrations of Fe, Mn, and Sr in all soil samples significantly exceed the content of other heavy metals. Compared to other studied elements, all the soil samples have the lowest concentrations of Co and Pb. In descending order of their contents in all soil samples, these five elements are arranged as follows: Fe > Mn > Sr > Pb > Co.

If we organize heavy metals in descending order of their concentration in leaf samples, we can observe that for the control leaves, the order is Fe > Cu > Zn = Mn > Cr = V > Ni = Sr. For soil samples contaminated with 1ml of crude oil, the order is Fe > Cu > Zn = Mn > V > Ni > Cr > Sr. The order in soil samples contaminated with 10 and 30 ml of crude oil is the same as before, with Fe > Cu > Zn > Mn > V = Ni > Cr > Sr. Therefore, in leaf samples, the content of Fe, Cu, Mn, and Zn is significantly higher than other heavy metals, while the contents of Cr and Ni are less than others. Co and Pb are not concentrated in the leaves at all.

Table 2 presents the BAC (Bioaccumulation factor) of heavy metals in V. faba leaf samples. The study proposes four categories of heavy metal accumulation: plants that do not accumulate heavy metals (<0.01), low accumulator plants (0.01-0.1), moderate accumulator plants (0.1-1), and high accumulator/hyper-accumulator plants (1-10) [28]. The results show that *V. faba* is not a hyperaccumulator for any of the studied heavy metals.

When heavy metals are arranged in increasing order of their BAC values in leaf samples, we obtain the following order: Sr < Fe < Mn < Cr < Ni < V < Zn < Cu.

Table 2. Bioaccumulation coefficient (BAC) of heavy metals in the leaf samples of *V. faba*.

Soil samples	BAC									
	Cr	Mn	Fe	Co	V	Ni	Cu	Zn	Sr	Pb
control soil	0.22	0.09	0.07	-	0.40	0.25	1.25	0.85	0.03	-
S-1	0.15	0.08	0.05	-	0.50	0.33	1.00	0.72	0.03	-
S-10	0.14	0.07	0.06	-	0.40	0.27	0.76	0.69	0.03	-
S-30	0.14	0.08	0.06	-	0.36	0.27	0.62	0.65	0.03	-

Sr, Fe, and Mn have low BAC values (BAC < 0.1), although the concentrations of these elements in soil samples are much greater than other heavy metals. *V. faba* is a low accumulator of Sr, Fe, and Mn.

BAC values of Cr, Ni and V remained below 0.5 (0.1 < BAC ≤ 0.5). The BAC values of Cr vary between 0.14 -0.22, while Ni varies between 0.25 -0.33, and V varies between 0.36 -0.40.

In general, Cu and Zn have the highest BAC values (0.6 < BAC ≤1.25) compared to other metals. The BAC values of Zn vary between 0.65-0.85, while the BAC values of Cu range from 0.62 to 1.25. For both elements, the BAC in control leaves is greater than in leaf samples grown in soils exposed to different concentrations of crude oil. *V. faba* is a moderate accumulator of Zn and Cu.

The results of the study of the impact of soil crude oil pollution on the FA indicators of *V. faba* leaves are given in Table 3. As the degree of contamination of soil samples with crude oil increases, the value of FA indicators also increases.

Table 3. The fluctuating asymmetry indicators of the leaves of *V. faba* grown in different soil samples.

Soil samples	BD $x \pm m_i$ (sm)	FAC	D
control soil	0.93±0.05	0.014	0.54
S-1	1.28±0.05	0.025	0.68
S-10	1.61±0.06	0.054	0.73
S-30	1.97±0.11	0.069	0.81

Compared to the control soil sample BD value of leaves in soil samples contaminated with 1, 10, and 30 ml of crude oil increases by 1.37, 1.73, and 2.11 times respectively. Similarly, the FAC value increased by 1.78, 3.85, and 4.92 times respectively. Additionally, the D value increased by 1.25, 1.35, and 1.50 times respectively in soil samples contaminated with 1, 10, and 30 ml of crude oil when compared to the control soil sample. An increase in FA indicators reflects phenotypic changes in leaf morphometric traits. This variation of FA indicators values manifests disturbance of developmental stability of the organism. An increase in the degree of soil contamination with crude oil leads to an increase in developmental instability in *V. faba* leaves.

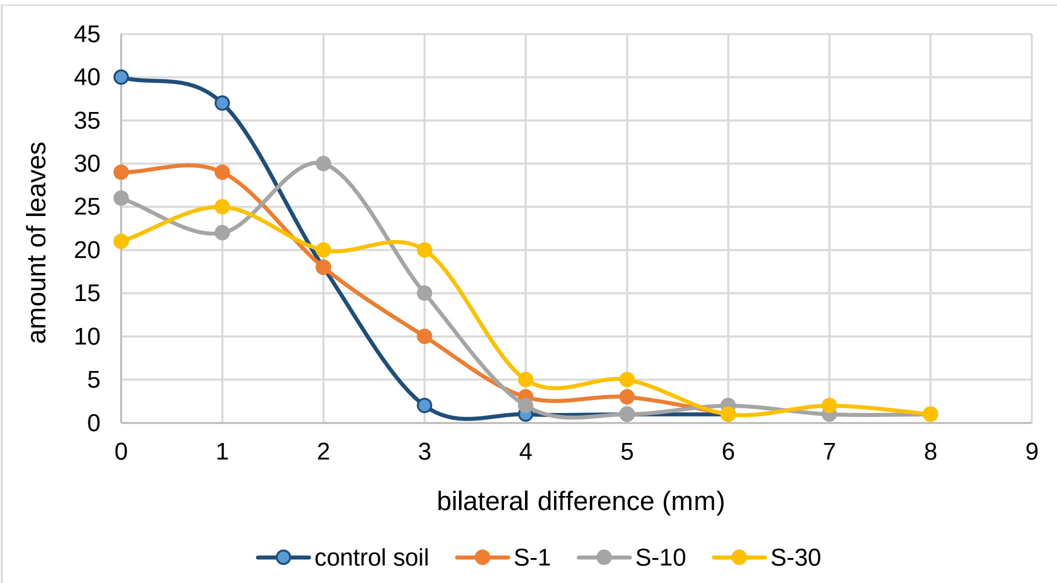

Fig. 3. Graphical representation of biological difference values within the studied trait.

Based on the data presented in Fig. 3, it can be seen that in the control leaf sample, asymmetry for the analyzed bilateral trait is absent in 40% of leaves. However, in leaf samples taken from soils contaminated with 1, 10, and 30 ml of crude oil, the value of this trait decreases to 29%, 26%, and 21%, respectively. In addition, higher values of bilateral difference (7 mm and 8 mm) are observed in leaf samples of V. *faba* plants growing on soils contaminated with 10- and 30-ml crude oil. This indicates destabilization of ontogenetic processes during the development of V. *faba* under conditions of soil pollution with crude oil.

4. Conclusions

Based on the results described above, it can be concluded that an increase in crude oil contamination in the soil leads to a rise in heavy metal content present in the soil. This results in the destabilization of the ontogenesis of the V. *faba* plant, leading to an increase in the V. *faba* leaves' FA indicators. The concentration of heavy metals, particularly Cu and Zn, increases in the leaves of the V. *faba* plant due to crude oil pollution.

Regardless of changes in the concentration of other metal elements in the soil V. *faba* plant can act as a moderate accumulator of Zn and Cu and can potentially be used for phytoremediation to clean up contaminated soil. The data obtained demonstrate that V. *faba* plant is sensitive to crude oil pollution and can be useful as an indicator for soils polluted by crude oil. Based on this, we can conclude that the leaves of this plant are indicative of soil pollution and can be used as natural filters to manage and clean up the quality of the environment.

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