

PHYTOINDICATION: REACTION OF PLANTS TO THE OIL LIGHT FRACTIONS

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ABSTRACT: The study of plant response to the lighter fractions of petroleum products. Place and Duration of Study: M.Auezov South Kazakhstan State University, Institute of Ecology and Biotechnology, between May 2012 and September 2014. We investigated 7 types of oil products (4 grades petrol, toluene, benzene, xylene) and 20 frequently encountered plant species in the south of Kazakhstan (from *Poaceae* -6, *Fabaceae* -8, *Brassicaceae* -4, *Asteraceae* -2) were used. We studied the morphometric changes and the percentage of germination of seeds of plants. It has been found that the light fractions of oil products have acute toxic effects on all plants. Grade AM-96 petrol (A – car, M- measuring the octane number) and xylene have been proven to be the most toxic among the studied oil products, including petrol of various standards, xylene, toluene, and benzene. The reactions of the studied species of plants to certain types of oil are differentiated, and among the studied species of plants, the representatives of the mustard family proved to be the most sensitive to the effects of light fractions of oil products, while representatives of the grass family were sensitive to the effects of toluene, benzene and xylene. The most resistant species are cultivated maize, kidney bean, cultivated chickpea, long-staple cotton, *Helianthus annuus*, *Cynodon dactylon* and alfalfa.

KEYWORDS: Seed Vigour, Morphometric Parameters, Test Plants, Grades Petrol

INTRODUCTION

It is known that oil and oil products entering the environment cause mixed reactions in biological objects. There is no consensus among researchers regarding the toxic effects of oil on plant organisms. Many researchers tend to believe that crude oil itself is not toxic to plants but that its negative effect occurs through the deterioration of the water-air regime of the soil, which creates conditions for the development of phytopathogenic soil microflora whose toxins, combined with reduced physiological factors, cause the death of plants (*Delille and Pelletier*, 2002; *Davis et al.*, 2002; *Banks et al.*, 2003; *MacKinnon and Duncan*, 2013). However, the results of a number of researchers have found that the negative impact of oil on plants depends on its concentration in the soil—1-2% content stimulates the growth and development of many types of plants, whereas higher concentrations inhibit these processes (*Merkel et al.*, 2004; *Mohsenzadeh et al.*, 2009). Light fractions of oil products bled from crude oil fill soil pores and have a toxic effect on the root systems of plants; as a result, the pace of the metabolic processes of the plant organism are sharply reduced (*Liste and Alexander*, 2000; *Wyszkowska et al.*, 2002; *Sharonova and Breus*, 2012). Such criteria regarding the morphometric features of plants as plant germination, growth rate, biomass of above-ground organs, root biomass and seed production are reduced with increasing concentrations of oil in the soil (*Adam and Duncan*, 2002; *Potashev et al.*, 2014). Oats were found to be more resistant than corn, perennial grasses,

alfalfa and barley to the toxic components of oil (Unkefer et al., 2001; Tang et al., 2010). It has also been found that the degree of inhibition of plant growth and development is proportional to the dose of oil (Šimonová et al., 2005; Wyszowski and Wyszowska, 2005). Furthermore, it is necessary to consider the role of the rhizosphere microflora in securing the stress resistance of plants against oil effects (Binet et al., 2000; Tesar et al., 2002; Zhang et al., 2010).

The results of previous studies (Bishimbayev and Issayeva, 2013) contrast somewhat with these results due to differences in soil types and the choice of herbal test objects. This contradiction and agribusiness specialization of the South Kazakhstan region were the rationale for the study of the reaction of industrial crops of plants to soil contamination based on the effects of different light fractions of oil products. A detailed study of the phytoindicational properties of high vascular plants will help to develop an application methodology for regional and theoretical lists of phytoindicators in the form of scales of tolerance.

MATERIALS AND METHODS OF INVESTIGATION

Oil products

Grades AM-80, AM-85, AM-92, AM-96 petrol, toluene, benzene and xylene were used.

The test plants

The test plants included representatives of the cultivated grass family such as spring barley, spring wheat, oat, millet broomcorn, and cultivated maize; from the *Poaceae* family—scutch grass (*Cynodon dactylon*), annual bluegrass (*Poa annua*), bulbous bluegrass (*Poa bulbosa*), brome (*Bromis tectorum*), Aegilops cylindrical (*Aegilops cylindrica*) and mouse barley (*Hordeum murinum*); from the *Fabaceae* family—cultivated mung bean, cultivated chickpeas, lentils, kidney bean, alfalfa, red clover, white clover, *Trifolium hybridum*; from the *Brassicaceae* family—watercress salad, cultivated radish, mustard, radish; and from the *Asteraceae* family—*Helianthus annuus*, safflower planting.

Terms of the laboratory experiments

In previous laboratory studies on the effect of oil and petroleum products on the plant, a hydroponic system was used where the seeds were planted at a depth of 2.5-4.0 cm in plastic cups with 200 g of calcined and sifted sand. The plants were fertilized with the “Kemira flower” mineral complex. Oil and oil products were introduced in amounts of 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8 volume % (where 1 volume % -1 g oil per 100 g of sand).

Seed vigour

Assessments of seed vigour were conducted on the 4th, 7th and 10th days, expressed as the percentage of germinated seeds.

Morphometric parameters of the test plants

The reactions of the plants to the oil and oil products were determined by the parameters such as the height of the plants, root length and biomass relative to the control.

RESULTS AND DISCUSSION

Influence of different grades of petrol to plants

In studying the effects of different concentrations of grades petrol such as AM-80 and AM-85 on the seed germination, growth and development of grass plants, it was found that the concentrations of $0.1 \pm 0.05\%$ and $0.2 \pm 0.05\%$ had a stimulatory effect on plant growth but an inhibitory effect on root development. Increasing the oil products concentration to $0.3 \pm 0.05\%$ and higher led to acute toxic effects. The concentration of $0.4 \pm 0.01\%$ was fatal for all plant species. The most sensitive was spring barley, as even the $0.1 \pm 0.05\%$ concentration caused a decrease in seed germination to $20.7 \pm 1.3\%$, while spring wheat and oat turned out to be more resistant, decreasing seed germination only slightly at a $0.3 \pm 0.04\%$ concentration. In millet broomcorn, seeds germinated at a $0.6 \pm 0.01\%$ concentration, and the most resistant was cultivated maize at a $0.9 \pm 0.04\%$ concentration.

A further increase in the concentration petrol standards in the soil led to the death of seeds. Grades petrol AM-92 and AM-96 showed acute toxic effects even at concentrations of $0.1 \pm 0.05\%$ and $0.2 \pm 0.05\%$. The fatal concentration for spring barley, spring wheat, oat and millet broomcorn was $0.3 \pm 0.05\%$, while for cultivated maize the value was $0.5 \pm 0.05\%$.

The study of the reaction of wild grain crops to various standards of petrol showed that scutch grass (*Cynodon dactylon*) and annual bluegrass (*Poa annua*) were more stable than bulbous bluegrass (*Poa bulbosa*), cheat grass (*Bromis tectorum*), Aegilops cylindrical (*Aegilops cylindrica*), or mouse barley (*Hordeum murinum*), as all petrol standards in all of these species were fatal to seed germination at a $0.3 \pm 0.02\%$ concentration. Seeds of scutch grass and annual bluegrass are stable in a $0.1 \pm 0.01\%$ contamination of the soil by the light fractions of oil products, and at a concentration of $0.2 \pm 0.01\%$, the decrease in seed germination does not exceed $40.0 \pm 2.3\%$. The fatal concentration for these plants was $0.3 \pm 0.02\%$ of grades petrol AM-80, AM-85, AM-92 in the soil content and $0.2 \pm 0.01\%$ for grade AM-96 petrol.

Leguminous crops proved to be more resistant to contamination of the substrate by grade AM-80 petrol, with the fatal threshold being lower, at $0.6 \pm 0.05\%$. In the case of contamination of the substrate by grade petrol AM-85, beginning from the concentration of $0.2 \pm 0.01\%$, a noted decrease in the morphometric parameters of plantlets compared to the control variant of $65.6 \pm 4.2\%$ occurred. Grades petrol such as AM-92 and AM-96 have a toxic effect on the germination of seeds, and a concentration of $0.3 \pm 0.01\%$ was fatal for all cultures. This result revealed a strong inhibition of the growth of the roots and stems of the plants. In order of increasing sensitivity to the effects of petrol standards, the studied cultures appear to have settled in the following order: cultivated mung bean → cultivated chickpea → cultivated lentils → kidney beans.

In studying the effects of different petrol standards on the development of perennial legumes, it is found that leguminous crops and perennial legumes do not significantly differ in their degree of resistance. Alfalfa proved to be more resilient to the effects of grades petrol among the studied perennial legumes. The highest sensitivity was found in white clover and clover hybrid. It may be noted that the consistent pattern in the plant reaction to the contamination of the substrate by different standards of petrol was maintained. AM-92 and AM-96 are the most toxic of the analysed petrol standards.

In studying the effects of various petrol standards on the representatives of the mustard family, it was found that, regardless of the petrol standard, a concentration higher than $0.01 \pm 0.001\%$

causes a total loss of watercress seeds, which are commonly used in bioindication object phytotesting. A study of the reaction of cultivated radish, mustard and radish showed the greatest stability of the last plant, where 5.0% of seeds sprouted even with $0.25 \pm 0.5\%$ the AM-85 content in the soil. Although seeds do sprout at a concentration of 0.1% and $0.2 \pm 0.01\%$, the seedlings are weak and quickly turn yellow and die on the 5th-6th days. The root system is underdeveloped, additional roots are few. The studied species of the mustard family, in order of increasing resistance to the effects of petrol standards, have adopted the following order: watercress salad → cultivated radish → saperda mustard → garden radish.

In studying the reactions of industrial crops such as the cultivated beet, *Helianthus annuus*, safflower and cotton plant to soil contamination by different petrol standards, it was found that cultivated beet was sensitive to even low concentrations of all investigated standards of petrol.

All types of experiments indicated a strong inhibition of the growth and development of shoots within five days of the experiment. The seed germination decline to $20.3 \pm 2.6\%$, $24.5 \pm 3.1\%$ and $28.5 \pm 2.9\%$ at observed concentrations of grades petrol AM-80 and AM-85 are higher than $0.7 \pm 0.07\%$ in the cotton plant, safflower and sunflower.

A further increase in the concentration of petrol led to the complete destruction of seedlings of cultivated safflower, a decrease in seed germination of the cotton plant to 85.9 ± 10.7 and of the sunflower to $60.6 \pm 9.6\%$. This plant looked wilted and fell behind in terms of growth compared with a control group of plants to $70.6 \pm 7.2\%$.

Plant reaction for toluene, benzene and xylene

During the study of the reactions of phyto test groups of plants to the effects of toluene, benzene and xylene, it was found that starting from a concentration of $0.2 \pm 0.01\%$, toluene and benzene have an inhibitory effect on the seed germination of all studied species. It was also found that xylene is more toxic than benzene and toluene. Industrial crops proved to be the most stable to the effect of toluene, as concentrations of $0.1 \pm 0.01\%$ to $0.3 \pm 0.01\%$ had not significant effect on the seed germination of cotton plant, sunflower and safflower. The significant part of the seeds did not produce seedlings with increasing concentrations up to $0.5 \pm 0.05\%$. In addition, with the increasing concentration of the toxicant, a decline in the growth of seedlings and plant biomass has been observed. Fatal concentrations of toluene and benzene in the analysed species were $0.7 \pm 0.07\%$. Leguminous crops studied with regard to the degree of resistance to the effect of toluene and benzene are located in a number of the following: beans, chickpeas and lentils. At the same time, a decrease in the seed germination of kidney beans and chickpeas to 0.6% for various concentrations of toxicants was not observed. A significant decrease in the plant biomass of kidney bean of $26.8 \pm 2.5\%$ at a concentration $0.5 \pm 0.05\%$ was observed.

The increasing concentrations of benzene and toluene to $0.7 \pm 0.07\%$ proved to be fatal to chickpea and, at $0.9 \pm 0.1\%$, to the kidney bean. Among the studied leguminous crops, cultivated lentils proved to be less resistant to benzene and toluene-tested lentils, so the $0.3 \pm 0.01\%$ concentration of benzene and toluene reduced seed germination 96.5 - 94.3%, the biomass of plants to 88.6 - 89.6%, and a concentration of $0.5 \pm 0.05\%$ was fatal to them. The studied concentrations of toluene, starting with $0.3 \pm 0.01\%$, exerted an acute toxic effect on all types of crops.

At the same time, cultivated corn, which was more resistant to the petrol standards, showed a sensitivity to $0.4 \pm 0.01\%$ toluene, in which seed germination was reduced $48.6 \pm 4.6\%$, and the reduction in biomass plants was $78.7 \pm 6.6\%$

No plant germinated at $0.5 \pm 0.05\%$ toluene, revealing that the reaction of the phyto test with plants is similar to the reaction of xylene with toluene. Thus, it was determined that the fatal concentration of xylene in the soil was $0.2 \pm 0.02\%$ for barley and oats and $0.4 \pm 0.02\%$ for maize.

The decrease in germination of seed and biomass was directly correlated with increasing concentrations of xylene in the soil. The study of the reaction of leguminous crops to xylene showed that the most stable was the kidney bean, which at a $0.4 \pm 0.03\%$ decrease in seed germination was $45.5 \pm 3.5\%$, and the fatal concentration was 0.5 ± 0.02 . However, inhibition of the growth of the young seedlings was observed. Seedlings appeared three days later than in the control variant and chronically lagged behind in growth, which was significantly reflected in the difference in plant biomass. The concentrations of $0.1 \pm 0.02\%$ and $0.2 \pm 0.02\%$ had a strong inhibitory effect on chickpeas and lentils, and the fatal concentration was $0.3 \pm 0.02\%$. Similar results were obtained when studying the resistance of industrial crops to xylene. The seed germination of safflower and sunflower decreased to $85.4 \pm 4.5\%$ and $79.9 \pm 6.5\%$ at 0.1% and 0.2% concentrations of xylene, respectively. It was found to decrease the seed germination of cotton plants to $85.4 \pm 8.5\%$ and the biomass of seedlings to a 0.3% concentration of xylene. The concentration of 0.4% was fatal to all examined plants.

The reactions of perennial legumes to the effects of toluene, benzene and xylene were similar to their reactions to different standards of petrol; thus, clover and clover hybrid are sensitive to a $0.05 \pm 0.005\%$ concentration of oil products. Decreased seed germination is $98.9 \pm 0.5\%$. It was found that among all the studied plants, alfalfa was the most resistant to the given group of oil products, and the total destruction of the seed occurred at a $0.3 \pm 0.02\%$ concentration. The study of the reactions of representatives of the mustard family also revealed plant species that differed in sensitivity to the analysed group of oil products. The laboratory studies showed a high sensitivity of traditional herbal test objects—cress, whose seeds died out at $0.05 \pm 0.005\%$ soil contamination with xylene, toluene and benzene. Radishes produced seedlings even at 0.3% toluene but died at a concentration of xylene above $0.05 \pm 0.005\%$ and at a $0.2 \pm 0.02\%$ concentration of benzene.

CONCLUSION

It was found that the light fractions of oil products have acute toxic effects on all plants. Grade AM-96 petrol and xylene proved to be the most toxic among the studied oil products, which included petrol of various standards, xylene, toluene, and benzene. The reactions of the studied species to certain types of oil products are mixed, while among the studied plants species, the representatives of the mustard family proved to be the most sensitive to the effects of light fractions of oil products, and representatives of the grass family are the most sensitive to the effects of toluene, benzene and xylene. The most resistant species are cultivated maize, kidney bean, cultivated chickpea, long-staple cotton plant, *Helianthus annuus*, *Cynodon dactylon* and alfalfa.

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