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RESEARCH ARTICLE

An *In vivo* Investigation of the Effects of Some Heavy Metals on the Development of Beans (*Phaseolus vulgaris* L.)

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HIGHLIGHTS

> The effects of some heavy metals were researched on the plant growth of the seeds of İspir bean (*Phaseolus vulgaris* L.)
> The bean plant showed an unhealthy growth and was adversely affected from heavy metals

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ABSTRACT

The effects of metals on the ecological system are very important, and their presence on certain limit values has negative effects on the living things. In particular, it has been determined that structural and functional changes are present in plants exposed to metals at high rates. The accumulation of heavy metals in the environment is a serious problem. In this study, the effect of heavy metals such as Co^{2+}, Ni^{2+}, Cu^{2+} and As^{3+} on the plant growth of the seeds of İspir bean (*Phaseolus vulgaris* L.), a species peculiar to the İspir region of Erzurum and well known by public, was investigated. The amount of metal ions was determined using spectrophotometric methods in all samples. As a result, it was observed that when the seeds were exposed to heavy metals, the plant showed an unhealthy growth and was adversely affected.

Contents

1. Introduction

Environmental pollution is the ecological harm that is caused by human beings that breaks the natural equilibrium in ecosystems. The pollution of solid, liquid and gaseous pollutants coming from various sources comes from the pollution of the environment with high accumulation in air, water and soil. We can classify the main types of pollution as air pollution, water pollution, soil pollution, noise pollution and radioactive pollution. Heavy metals that can be found in soil, water and air at different rates lead to pollution after certain concentrations. Scientific studies have been shifted to this direction and subject to a lot of

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researches because heavy metals come at the top of polluting elements and this pollution is dangerous both for plant and human health. During these studies, scientists have tried to understand what sources of heavy metals are spreading around the environment, how they are present in the air, water and soil, what damages they cause to plants, animals and humans, and at what concentration they are toxic. It is known that metal ions and chlorides are toxic to living organisms and cause food contamination by entering the food chain [1, 2].

Metal ions such as lead, zinc, copper, cobalt, cadmium, chromium, nickel, arsenic, mercury and silver are important for their live systems and environmental health due to their permanent harmful effects and they are highly toxic even if they exceed a certain limit [3–5]. In addition, cancers called ages and pus are also proven by their studies [6]. This is the reason why they are regarded as the most serious environmental problem. Most of these elements; people go into the body secretly (through water, food chain, etc.) and cause serious illnesses without ever knowing it [7].

In this study; The effects of heavy metals such as Co^{2+}, Ni^{2+}, Cu^{2+} and As^{3+} on the development of bean seed (*P. vulgaris* L.), a species specific to the Erzurum-İspir region, and the amount of metal ions metabolized and absorbed during seed development were investigated.

2. Materials and Method

2.1. Bean seed

Beans, which are an agricultural plant, are considered as important nutrients. Therefore, in our research plant seeds of İspir bean grown in İspir province of Erzurum were used as plant material. The seeds were obtained from a local market in Erzurum and were discovered by botanists in the Department of Biology at Ataturk University, Faculty of Science.

2.2. Seed germination

Bean (*P. vulgaris* L.) seeds were planted in a plastic sowing cabinet, which was washed in and swollen environment was soaked with pure water and cotton was laid. After the planting, the seeds were allowed to germinate for 1 week.

At the end of this run, the roots of the homogeneously selected bean seeds were laid down after the roasting process in such a way that they were entirely contained in 0.1% prepared metal chloride solutes ($CoCl_2$, $NiCl_2$, $CuCl_2$ and $AsCl_3$).

The prepared cultures were allowed to stand for 7 days. At the end of 7 days, samples were taken from each of them at intervals of 3 days and the amount of metal absorbed by the seeds and the growth of the seeds were examined. All experiments are given in Figure 1. Pure water was used as a control.

A B C D

Figure 1. Beans and controls grown in different solvents (A: Ni^{2+} solution, B: Cu^{2+} solution, C: Co^{2+} solution, D: As^{3+} solution)

2.3. The amount of metal absorbed by the seeds

The amount of Co^{2+}, Ni^{2+}, Cu^{2+} and As^{3+} ions in the samples was determined spectrophotometrically. Blind pure water was used. Images of the assay are given in Figure 2

2.3.1. Determination of Nickel ($NiCl_2$)

The amount of nickel ion in the samples was done spectrophotometrically using 1mM dithizone solution (Figure 2). For this purpose; 1.5 mL of purified water was added to 1 mL of 1 mM dithizone solution and the sample used at 500 mL plant growth was incubated for 30 min at 30 °C in a water bath and the absorbance changes at 740 nm were recorded. Pure water was used as a blind sample [8].

2.3.2. Determination of Copper ($CuCl_2$)

The amount of copper ions in the samples was done spectrophotometrically using 1-amino-2-hydroxy-4-naphthalenesulfonic acid solution. For this purpose; the absorbance change was measured at 257 nm after 500 mL of sample was taken and 1.5 mL of 1 mM 1-amino-2-hydroxy-4-naphthalenesulfonic acid solution was added and incubated at 30 °C for 15 minutes. It was blindly read as pure water instead of sample [8, 9].

2.3.3. Determination of Cobalt ($CoCl_2$)

The amount of cobalt ion in the samples was done spectrophotometrically using the dithizone solution (Figure 2). For this purpose; 1 mL of 1 mM dithizone solution and 1.5 mL of purified water were taken and 500 mL of the sample was placed in the water bath for 30 minutes at 30 ° C and absorbance change was measured at 620 nm. Pure water was used instead of sample as a blind [10].

2.3.4. Determination of Arsenic ($AsCl_3$)

The amount of arsenic ion in the samples was determined spectrophotometrically. For this purpose; 0.5 mL samples were taken and 2 mL of KIO_3 was added followed by stirring for 2 min.

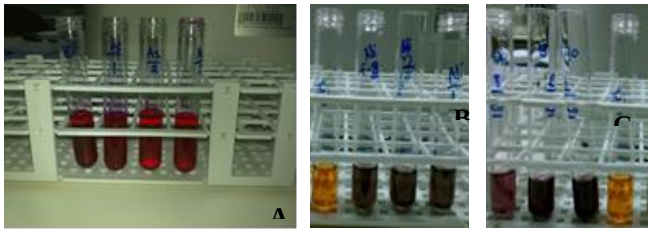

Figure 2. Spectrophotometric determination of metal ions in the samples (A: Ni^{2+} ion, B: Cu^{2+} ion, C: Co^{2+} ion, D: As^{3+} ion)

The pH of the medium was then set to 6.5. Mixture 2 mL of 0.2% Rhodamine B was added and waited for 20 min

before measuring the absorbance change at 556 nm. It was blindly read as pure water instead of sample [11].

3. Results and Discussion

Root and trunk lengths of bean seeds were measured and it was determined whether the roots were branched. In addition, their development and size were generally assessed and photographed (Figure 3).

Cu^{2+} *Ni^{2+}* *As^{3+}* *Co^{2+}* *Control*

Figure 3. Photograph of bean seeds grown in different metal ions.

Table 1 Comparison of bean seeds grown in different metal ions.

Seed	Control	$CoCl_2$	$AsCl_3$	$NiCl_2$	$CuCl_2$
Size	10 cm	7.0 cm	9.0 cm	9.0 cm	4.0 cm
Root	7.0 cm	3.0 cm	2.5 cm	4.0 cm	2.5 cm
Weight	2.75 gr	2.04 gr	1.24 gr	1.25 gr	0.64 gr

The development of seeds (*P. vulgaris* L.) seeds to which metal ions were applied from the obtained results was compared (Table 1). As can be seen from Figure 3 and Table 1, it was found that the control seed was more rooted and grown.

The seed growth rate was observed to be weaker than the control, although the root branch of the bean seed grown in the $CoCl_2$ solution was less than that of the control and the length of the seeds remained shorter and the seed body appeared healthy.

The bean seeds in the $AsCl_3$ solution were measured as 9 cm in length and close to the control, although their roots and body were weak and scarce.

Root branching, stem development and seeding of the bean seed grown in the $NiCl_2$ solution are very weak and weak, as shown in Figure 3.

Finally, it was determined that the root branching in the seed grown in the $CuCl_2$ solution is minimal and the growth is the least growing seed compared to the other heavy metal solutions.

Determination of the amounts of metal ions used by plants was determined spectrophotometrically and the results are given in Table 2. When the usage percentage of the copper (II), As (III), Co (II) and Ni (II) metal ions were determined within 1-5 days, the plant used the most Ni ions at the end of the 5th day and, at the same time. It was observed that the Ni^{2+} ion was present in the applied plant seed.

Table 2. Changes in the amounts of Cu (II), As (III), Co (II) and Ni (II) metal ions in the growth medium of plants.

Time	Amounts Removed			
	% Cu (II)	% As (III)	% Co (II)	% Ni (II)
Initial	100	100	100	100
Day 1	85.91	84.37	83.40	90.37
Day 3	88.82	92.12	85.75	90.48
Day 5	92.11	97.184	87.26	98.32

4. Conclusion

According to the findings, it was determined that bean (*P. vulgaris* L.) plant metabolizes heavy metals and is mostly affected by Ni^{2+} ion. In addition, metal ions absorbing plant is an unhealthy seed and is adversely affected as a result of measurements made are determined. It has been determined that plants such as seedling viability, heavy weight reducing toxic effects and water uptake are very effective in the life stages of heavy metals and have a negative effect on seed germination and prevention of healthy growth depending on the metal species.

It has been determined that the beans obtained from the obtained bees cannot show a healthy development and growth by being adversely affected by environmental conditions with heavy metal content.

In this study, the greatest danger for us is that vegetables and fruits that are raised in adverse environmental conditions and are present in our nourishment are dangerous to us. It is an indication that the heavy organisms entering the living organism through nutritional chain may be a factor in the formation of dangerous diseases.

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