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Study of *Anemone coronaria* L. (Manisa Tulip) Species, Symbol Plant of Manisa Province, for Biomonitor and Phytoremediation Purposes

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Abstract

Today, the use of plants in the cleaning (phytoremediation) of soils polluted with heavy metals and the detection of plants with accumulator properties are gaining importance day by day. However, due to the tolerance of plants against heavy metal toxicity, their ability to be bioindicators varies depending on the plant species, element type, duration of exposure to stress, and the structure of the tissue or organ exposed to stress. To date, many plants have been used in remediation, but there are few reports on the use of ornamental plants for remediation of polluted soils or as bioindicators.
In this study, based on this idea, accumulation of heavy metals of Cu, Cd, Pb, Zn were detected comparatively in 3 varieties (var. *alba*, var. *cyanea*, var. *coccinea*) of the *Anemone coronaria* species with atomic absorption spectrometry in the months of germination (March) and flowering (April) and in the soil they grow. As a result, it has been established that this species can be planted in Manisa to determine the soil pollution in the spring period, as a bioindicator, and to beautify the environment and it has been revealed that this species is a very important plant in terms of phytoremediation and as the symbol plant of Manisa.

Keywords: *Anemone coronaria*, Manisa, biomonitor, phytoremediation.

1. Introduction

Industry and technology negatively affect the ecological balance and harm nature and living things in various ways. Heavy metal pollution is one of the important harmful pollutants. Precautions should be taken to prevent living things from being damaged by heavy metal pollution in soils (1). Many methods and techniques are used to reveal the existing polluting elements in the areas to be studied for pollution. Today, the determination and monitoring of flora elements and environmental quality have become a very common and reliable method (2-4). Pollutants affecting the biotic and abiotic components of the ecosystem are accumulated by plants (sea creatures, lichens, fungi, bark, leaves, etc.) which are good indicators, in their bodies and it is reported that they provide information about environmental quality (2-5). It is known that heavy metal accumulation varies depending on the type of plant, the structure of the soil where it grows, climatic conditions, and especially the type and density of the pollutant (6, 7). Although some plant species disappear in the presence of very few pollutants, some plant species can survive at high concentrations. By examining the responses of plants to environmental factors from morphological, anatomical, and physiological aspects, the extent of pollution can be measured much more economically than physical and chemical measurements (2, 6, 8, 9).

Studies conducted in recent years show that heavy metal pollution, which increases depending on the density of fossil fuels and industry etc, has an ecotoxicological effect. In addition to these, it is stated that it has effects such as growth problems in plants, negative effects on quality factors, decrease in yield, acute and chronic poisoning in humans and animals such as the decrease in the biological activities of microorganisms in the soil (7, 8, 10).

Heavy metals spread easily to the environment through many activities especially as nuclear and thermal power plants, factories, flue gases, domestic waste, mining operations, traffic, pesticides, excessive fertilization, etc. In addition, outside of the respiratory tract, heavy metals are transmitted to humans by consumption of foods of plant and animal origin. Therefore, it is important to understand the soil-plant-animal relationships (11, 12).

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Members of the *Anemone* species, which belongs to the Ranunculaceae family, are found in the Northern Hemisphere and grow in temperate climates, and are found in high areas in the tropics (13, 14). *Anemone coronaria* L. species is of Mediterranean origin and is known as 'Manisa Tulip' or 'mountain tulip' in our country (15, 16). Morphological, anatomical, and palynological studies have been performed on *A. coronaria* species (17-22).

Members of the *A. coronaria* species are perennial plants that bloom in March and April (May) in Manisa as a harbinger of spring and are also used for decorative purposes. In this study, 3 varieties of *A. coronaria* species that spread on the Kırkağaç-Soma highway of Manisa were studied comparatively and were used as a bioindicator for the first time to monitor heavy metal accumulation. However, varieties of *Anemone coronaria* species, which attract attention with their trading feature in the Netherlands, have been proposed for the first time for phytoremediation and bioindicator-based use in this study.

This study will constitute a basis for future research on similar topics. However, it was carried out as the first stage of more comprehensive research on the same and different species planned to be carried out in other regions of Manisa.

2. Materials and Methods

3 varieties belonging to the *A. coronaria* species; Kırkağaç-Soma highway of Manisa province is a region that is packed with heavy vehicles due to the lignite coal transportation performed in the direction of İzmir and İstanbul. It is of particular importance that the Soma Thermal Power Plant is located 4-5 km from the area where the plants are collected. This locality was especially chosen because the pollution caused by the heavy traffic and the thermal power plant bears great importance for the people, plants, animals, and soil in the region.

The taxa used as examples and belonging to the Ranunculaceae family are *A. coronaria* var. *coccinea* (Hanry) Batt (red-flowered), *A. coronaria* var. *cyanea* (purple-flowered), and *A. coronaria* var. *alba* Goaty&Pens (white-flowered) (14). The plants were collected in the last week of March (24.03.2017), when they were non-flowering, and in the last week of April (24.04.2017) when they were flowering. On both dates, soil samples were taken from the same 10 different points of the area where all 3 taxa spread together and they were mixed. Soil sampling depth was determined as 0-15 cm since the tubers were very close to the surface of the soil. In March, tubers and leaves of taxa, in April, tuber, leaves, scapus, flowers, and in both months, soil samples were divided into groups to determine heavy metal (Cu, Cd, Pb, Zn) levels.

Plant specimens placed in paper envelopes were dried at 105 ºC for 24 hours. 1 gr was taken from the plant samples that lost their moisture completely, acid was added in the ratio of HNO_3: $HClO_4$ (4:1), and they were decomposed in the CEM MARS-5 ESP 1500 PLUS microwave sample preparation device. Soil samples were passed through a 2 mm sieve and dried at 105 ºC for 2-4 hours. Soil samples taken in 2 gr were decomposed in CEM Mars -5ESP 1500 PLUS microwave sample preparation device in HNO_3 : $HClO_4$ (3:1). All metal analyses were performed on the VARIAN 220 atomic absorption spectrometer (23).

3. Results and Discussion

When the heavy metal Cu, Cd, Pb, and Zn (ppm) accumulation levels measured in the varieties of *A. coronaria* species and in the soil samples of the area where these samples were collected are examined, the values of April were found to be higher than the values in March in both groups (except for Cu leaf and var. *cyanea* Pb leaf) (Tables 1, 2, 3).

Table1. Heavy metal distribution by months in soil samples

Month	Cu (ppm)	Cd (ppm)	Pb (ppm)	Zn (ppm)
March	2,71	could not be measured	18,96	5,89
April	3,81	1,01	27,10	11,14

Since March is not the flowering period of the plant and the plant cannot be determined in terms of varieties, tuber and leaf were evaluated. In April, the plant was divided into varieties and spectrometric measurements were made on tubers, stems, leaves, and flowers. The results obtained with the study are given in Tables 2 and 3.

Table 2. Heavy metal measurements of March (24.03.2017) in *A. coronaria* species

Parts of plants	Cu (ppm)	Cd (ppm)	Pb (ppm)	Zn (ppm)
Tuber	1,91 ±0,42	could not be measured	7,84 ±0,96	4,86 ±1,14
Leaf	9,57 ±1,25	could not be measured	9,51 ±1,88	3,46 ± 0,78

When the heavy metal distributions in March and April are evaluated in soil samples, it is seen that the amount of Cu and Pb goes up in April, and the amount of Zn increases about 3 times. However, it is noteworthy that Cd was immeasurably low in March and it increased at a value that could be measured in a spectrometer in April (Table 1).

According to the heavy metal measurements made with only leaves and tubers of *A. coronaria* species since the plant is flowerless in March, it is noteworthy that while Cu and Pb accumulation is higher in the leaf than in the tuber, Zn accumulation is higher in the tuber than in the leaf. However, the fact that Cd is too low to be measured in leaves and tubers is another important data for March. Since there were no flowers in the plant during this period, scapus could not be evaluated (Table 2).

Table 3. Heavy metal measurements of April (24.04.2017) in *A. coronaria* species varieties

***Anemone coronaria* varieties**	**Parts of plants**	**Cu (ppm)**	**Cd (ppm)**	**Pb (ppm)**	**Zn (ppm)**
Anemone coronaria var. *alba*	Tuber	2,16 ±0,39	0,52 ±0,18	10,70 ±2,44	7,15 ±1,61
	Scapus	1,12 ±0,22	0,38 ±0,07	11,10 ±2,98	6,40 ±1,15
	Leaf	1,08 ±0,37	0,49 0,10	10,20 ±1,95	11,12 ±3,36
	Flower	2,24 ±0,78	0,39 ±0,14	9,80 ±3,04	6,54 ±1,47
Anemone coronaria var. *cyanea*	Tuber	5,43 ±0,98	0,64 ±0,21	14,70 ±4,86	9,28 ±2,11
	Scapus	0,95 ±0,34	0,40 ±0,09	8,80 ±1,96	4,32 ±0,80
	Leaf	0,98 ±0,21	0,53 ±0,11	8,80 ±2,23	11,29 ±2,86
	Flower	2,31 ±0,37	0,41 ±0,08	10,20 ±1,87	8,79 ±2,16
Anemone coronaria var. *coccinea*	Tuber	4,39 ±0,77	0,69 ±0,23	9,80 ±2,55	9,83 ±1,62
	Scapus	1,21 ±0,41	0,57 ±0,12	10,15 ±3,16	5,31 ±1,03
	Leaf	1,14 ±0,31	0,73 ±0,22	12,35 ±3,81	11,84 ±2,98
	Flower	2,76 ±0,63	0,53 ±0,09	9,50 ±1,11	9,22 ±1,52

When Table 2 and Table 3 are compared, although it will be noticed that there is a tendency towards an increase between two months in general, it is interesting that the amount of Cu measured in the leaf of the *A. coronaria* plant in March is 9,57 ppm, it shows a significant decrease in April in var. *coccinea* (1.18 ppm) > var. *alba* (1.08) > var. *cyanea* (0.98 ppm) and while other heavy metals increased in the other parts, Pb decreased in April in var. *cyanea* (8.80 ppm) compared to March (9.51 ppm) in the leaf. It can be indicated that the reason for this is that heavy metals are very effective and accumulate excessively in March when the leaf is in the sprouted state. Pb, on the other hand, can be stated to be less effective on the leaf in var. *cyanea* taxon. (Tables 2, 3).
In the April evaluations, *A. coronaria* species could be studied comparatively with its 3 varieties: var. *alba*, var. *cyanea* and var. *coccinea*.

If all three taxa of tuber are compared, var. *cyanea* has distinctively higher values for Cu and Pb than the other 2 taxa. However, when var. *alba* (except for Pb value) is evaluated, it contains the lowest amounts of heavy metals in tuber (Table 3).
var. *cyanea*-tuber (Cu) > var. *coccinea* (Cu) > var. *alba* (Cu)
var. *cyanea*-tuber (Pb) > var. *alba* (Pb) > var. *coccinea* (Pb)
var. *coccinea*-tuber (Cd, Zn) > var. *cyanea* (Cd, Zn) > var. *alba* (Cd, Zn)
When scapus is evaluated in general, var. *coccinea* has the highest values in terms of Cu and Cd and var. *alba* has the highest values in terms of Pb and Zn. In addition, var. *cyanea* contains the lowest values for all heavy metals (Table 3).
var. *coccinea*-scapus (Cu, Cd) > var. *alba* (Cu, Cd) > var. *cyanea* (Cu, Cd)
var. *alba*-scapus (Pb, Zn) > var. *coccinea* (Pb, Zn) > var. *cyanea* (Pb, Zn)
When a comparison is made in terms of leaves, var. *coccinea* taxon was observed to accumulate in higher amounts than all other taxa in terms of all heavy metals. Also, when var. *alba* is compared to var. *cyanea*, it will be seen that Cu and Pb are present in higher amounts in var. *alba* while Cd and Zn are in higher amounts in var. *cyanea* (Table 3).
var. *coccinea*-leaf (Cu, Pb) > var. *alba* (Cu, Pb) > var. *cyanea* (Cu, Pb)
var. *coccinea*-leaf (Cd, Zn) > var. *cyanea* (Cd, Zn) var. *alba* (Cd, Zn)
When flowers are evaluated, in terms of Cu, Cd, and Zn var. *coccinea* accumulated in the highest amount, which was followed by var. *cyanea* and var. *alba* accumulated in the lowest amount. In terms of Pb, it is seen that var. *cyanea* flower contains the highest amount of heavy metal (Table 3).
var. *coccinea*-flower (Cu, Cd, Zn) > var. *cyanea* (Cu, Cd, Zn) > var. *alba* (Cu, Cd, Zn)
var. *cyanea*-flower (Pb) > var. *alba* (Pb) > var. *coccinea* (Pb)

4. Discussion

Nerium indicum Mill, *Phragmites australis* Cav., *Zephyranthes candida* (Lindl.) Herb., *Cynodon dactylon* (L.) Pers., *Alternanthera philoxeroides* (Mart.) Griseb., *Chenopodium rubrum* L., *Aster subulatus* Michx, and *Brassica chinensis* L. species were used in a study conducted in polluted areas to reveal plants that may be suitable for bioindicator and/or phytoremediation studies. Given the fact that they are from different families, successful results have been obtained (24). The *A. coronaria* species used in our study is a plant suitable for being a phytoremediation and bioindicator plant, representing the Ranunculaceae family, with its capacity to absorb Cu, Cd, Pb, Zn heavy metals from the soil according to the months and organs.

The study conducted on industrial pollution and urban pollution using *Acer negundo* L. and *Platanus orientalis* L. in Antakya and ours are similar studies. As a result of the research conducted in Antakya, it was seen that the plants with high Cu and Al content were located on the city street, while the plants with high Cd, Pb, and Zn content were found to be on the industrially developed side of the city (12). Since the location chosen in our study is on a very busy road and is close to the Soma Thermal Power Plant, it also has the characteristics of the two locations selected in the study conducted in Antakya. However, Cu, Cd, Pb, and Zn heavy metals examined in our study were grouped among themselves according to plant parts, soil and months, and a detailed study specific to the spring season of Manisa province that could be repeated every year was created.

A study was conducted on the root length inhibitory effect of Cu, Cd, Pb, Zn, and Fe heavy metals, photosynthetic pigment content, and the effect of metal accumulation in roots and sprouts in *Sinapis alba* L. species. The study revealed that all heavy metals except Fe accumulated more in plant roots than in plant sprouts (25). Within the scope of this study, if the heavy metal accumulations in A. coronaria tubers and other parts are examined comparatively (Table 3), it is concluded that there was a remarkable amount of heavy metal accumulation in other parts of the plant as well, prominently in tubers. These results clearly show that the plant is an accumulator with every organ and is suitable for use in phytoremediation.

Cu, Cd, Pb, Zn, Ni, and Fe heavy metal levels on *Rosmarinus officinalis* L. samples and soils taken from various locations in Jordan were evaluated by atomic spectrometry. The plant was examined in three groups as leaves, flowers, and stems. Spinach leaves and ryegrass were used as reference materials and as a result, a high level of remediation was achieved (26). The plant A. coronaria, which we recommend as a phytoremediation and biomonitor plant used in this study, can also be used as a reference plant for other plants grown in Manisa in the spring season.

Salix phylicifolia L., *Salix borealis* Fr., *Carex rostrata* Stokes, *Eriophorum angustifolium* Roth, *and Phragmites australis* Cav. taxa were analyzed for the collection and transport of Cu, Cd, Pb, Zn, and As heavy metals from the soil. Among these species, only *Salix* species with Cd and Zn accumulator in their sprouts and *Eriophorum angustifolium* with Pb accumulator were found to be toxic species for animal grazing (27). The *A. coronaria* species suggested as a bioindicator in this study is a plant suitable to be used to easily determine toxic species in Manisa, where the lands begin to green up in the spring period especially to determine toxic species for animal grazing, as given in the study above, in accordance with the comparison at the organ level.

According to a study on the transfer of some heavy metals from soil to vegetables (transfer factor) using 20 different vegetables in China in 2007, the rate of transfer from soil to plant was found in the direction of Cd > Zn > Cu > Pb > Hg (28). In *.A coronaria* taxa, the transfer rate of heavy metals varies. In this regard, interpretation can be performed, and the transfer direction of heavy metals in the organs in taxa can be put in order as follows (Table 3): var. *coccinea*, Cu > Cd > Zn > Pb, var. *cyanea*, Pb > Cu > Cd=Zn, var. *alba* Pb > Cu > Zn > Cd. As can be seen, when compared with the study of Zheng et al. (2007), *A. coronaria* taxa did not comply with this study and therefore, it is a study that can be evaluated for different purposes.

Harvey (1971) stated that there are anthocyan-type substances in *A. coronaria* species, which are cyanidin, delphinidin, and pelargonidin (29). In this case, pelargonidin is found in red, delphinidin in blue, and cyanidin in the pink-purple variety. It can be indicated that Cu, Cd, and Zn accumulation in the flower exists in var. *coccinea*>var. *cyanea*>var. *alba* direction in the flower due to the effect of anthocyan-type substances found in *A. coronaria* taxa. It can be stated that it is in the var. *alba* direction. However, it is seen that the effect of anthocyanin substances on Pb accumulation is different from that of other heavy metals (Table 3).

In a review study on the toxic effects of Cu, Cd, Pb, and Zn heavy metals on vascular plants, it has been revealed that Zn is the least toxic heavy metal. However, no amount has been given in the literature in terms of toxic concentration for Cu, Cd, Pb, and Zn heavy metals in the soil (30). For this reason, although it is avoided to make any comments about the toxic concentration of our study area, we think that these evaluations should be updated with the increase in environmental pollution resulting from the developments such as today's increasing population, developing technology, and factories built, etc.

Table 4. Soma Station air quality index

Sample collecting days	**PM_{10} µg/m^3**	**SO_2 µg/m^3**	**CO µg/m^3**	**NO_2 µg/m^3**	**NOX µg/m^3**
24.03.2017	118,75	77,75	352,90	-	-
24.04.2017	48,02	13,47	10,69	13,27	32,56

It is a known fact that the most important sources of pollution in Soma-Kırkağaç are the emissions from Soma Thermal Power Plant, heating activities in residences, construction, and traffic based on heavy coal transportation. There are 2 stations in Manisa, one in the center and the other in Soma that measure air quality and collect daily data. The air quality data of the Ministry of Environment, Urbanization and Climate Change Soma Station for the study location and the days the samples were collected are given below (31).

In addition to PM_{10} (particulate matter) pollution, SO_2 (sulfur dioxide) values in Soma reach levels that threaten public health, especially in winter, and they always create pollution (32). As it is understood from this research, it is a known fact that the air pollution in Soma district is intense and Kırkağaç district, which is a district 10 km away, is adversely affected by the air pollution caused by the Soma Thermal Power Plant. However, as can be seen in Table 4, it is clear that the differences between the pollution levels in March and April in Soma district are high. In addition, although there are varying data on air pollution in Table 4, there is no information on heavy metals with carcinogenic effects, which pass through to the soil and from there to the plants, and thus to humans, with pollution-related emissions from traffic, etc. For this reason, *A. coronaria* species is a plant that should be brought to Manisa from a biological point of view due to its positive results in terms of its usability in phytoremediation due to its locality, perennial nature, and the fact that it is a bioindicator plant. As air quality indices are created, monthly herbal and soil-specific heavy metal indices can be created and shared with the public thanks to selected bioindicator plants.

5. Conclusion

The increasing traffic density of the Soma-Kırkağaç highway, coal activities, the development of residential areas, and the thermal power plant are the factors that increase pollution in the region. In this study, the selected plant was especially chosen because it is easy to understand in a short time whether it is easily affected by environmental pollution due to its short ground surface life span, its relationship with the soil, the relationship between soil pollution and the intake of this by the plant. However, in the study conducted, it is seen that there is a remarkable difference in terms of pollution between the spring period in March and April. Considering the increasing population, traffic, and the activity of the Soma Thermal Power Plant, it can be stated that this will change every year and that comparisons can be made easily with the bioindicator plant for these months. In addition, by taking into account this issue, local governments should take the necessary precautions.

This study, which was conducted to examine self-sown plants in nature and those also used as ornaments in the cleaning of environmental pollution, will constitute an important basis for the three-purpose-effective use of *A. coronaria* species for Manisa, which has economic and cultural importance, by demonstrating its ability to beautify the environment while being used for phytoremediation and biomonitor purposes.

Presentation of air quality indexes of regions and heavy metal accumulation data of selected seasonal plants with bioindicator properties on a half-monthly/monthly basis in addition to meteorological data in weather bulletins in mass media in big cities such as Manisa will enable working together in the solution of air and environmental pollution, in terms of right orientations regarding healthy nutrition and allow for healthy solutions to be put forward at the outset.

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Author's Contributions

Feyza Candan: Drafted and wrote the manuscript, performed the experiment and result analysis.

Ethics

There are no ethical issues after the publication of this manuscript.

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