Assessment of some urban ornamental plants in southern Iran revealed that they choose one of the two enzymatic or non-enzymatic antioxidants defensive strategies against heavy metals

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Abstract

Heavy metals (HMs) are one of the most important environmental contaminants. One of the effective approaches to reduce this contamination is phytoremediation. The concentration of ten heavy metals (HMs) including arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), tin (Sn), and zinc (Zn) were measured in ten ornamental plants consisting *Ficus religiosa*, *Ficus elastica*, *Syzygium cumini*, *Azadirachta indica*, *Clerodendrom inerme*, *Conocarpus erectus*, *Bougainvillea* sp., *Delonix regia*, *Dodonaea viscosa*, and *Phoenix* sp. grown in the city of Bushehr, located in southern Iran. The concentration of HMs was determined by ICP-MS. Some physiological parameters (relative leaf water, dry matter, vitamin C, pH, chlorophyll a, b, and total, carotenoid, anthocyanin, total flavonoid, total protein, total soluble carbohydrates, and proline), and antioxidant enzymes [peroxidase (POD) and superoxide dismutase (SOD)] activity were measured. *Bougainvillea* had greater amount of Zn (61.86 mg/kg D.W.), Cu (45.52 mg/kg D.W.), and Cr (14.71 mg/kg D.W.) than the other HMs. Greatest amount of Co (0.686 mg/kg D.W.), Fe (428.00 mg/kg D.W.), and Ni (6.44 mg/kg D.W.) was obtained in *C. erectus*. Also, *C. inerme* had maximum As (0.0087 mg/kg D.W.) and Sn (2.516 mg/kg D.W.). The highest percentage of dry matter (46.03%) in *Phoenix*, the highest amount of vitamin C (8.50 mg/100 g F.W.), chlorophyll b (7.63 µg/ml), and total flavonoid (3.92 M/g) in *S. cumini*, and the highest amount of chlorophyll a (14.61 µg/ml), total chlorophyll (21.86 µg/ml), carotenoid (20.83 µg/g F.W.), and anthocyanin (18.81 mg/100 g F.W.) in *F. elastica* were obtained. The POD activity (1.462 and 1.214 U/g F.W./min) was highest in leaves of *C. inerme* and *F. elastica* containing the highest concentrations of As, Sn and Pb, respectively. The SOD activity was high in these two species. Synthesis of osmolytes increased under Co and Sn stresses.

D. viscosa was found to contain most total soluble carbohydrates (24.94%) and proline (1.14 µM/g F.W.) concentration compared to other species. The selected plant species followed a defensive strategy (enzymatic or non-enzymatic) against excess of HMs. Overall, shrubs were much more suitable than trees for absorption of HMs, and *C. inerme*, *F. elastica* and *Bougainvillea* are the best plant species in this connection, respectively.

Introduction

Urban green spaces are a part of open spaces often made artificial, particularly dominated with cover plants, grasses, trees, and shrubs. Plants, especially trees and shrubs, can partially reduce the risk of pollutions caused mainly by heavy metals (HMs) and hazardous gases (Delaman and Farahmand 2017; Ghafari et al. 2020). The selection of suitable ornamental plants that are adapted to the urban area is the key to success in developing a sustainable green space (Ghafari et al. 2020). The main priorities for choosing plants in urban green spaces with extensive traffic are their ability to reduce environmental pollution without injury, architecture, beauty, performance, adaptation to different soils and climate, growth, being local, resistance to pests and diseases and ecological and aesthetic needs of each city or town (Cameron et al. 2012; Ghafari et al. 2020).

HMs include metals and metalloids with atomic density more than 4 g/cm^3 or 5 times or more than 5 times larger than water (Appenroth, 2010). According to these criteria, copper (Cu), manganese (Mn), lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), arsenic (As), silver (Ag), tin (Sn) and platinum (Pt) are HMs. Some of these HMs, such as Cu, Fe, Ni, and Zn are essential elements (micronutrients) for plants (Asati et al. 2016). A HM is not toxic in *per se* and is only toxic when its concentration in the plant cell is higher than the threshold level (a dose that causes the effect), which in this situation is called HM (Appenroth 2010). The gap between the essential concentration and the toxic concentration of some metals in plants is very low. This has attracted the attention of scientific associations toward the effects of HMs on plants and their critical roles in sustainable agriculture (Kaur and Garg 2021). Pollution of soil and water resources with HMs can lead to the absorption and accumulation of these elements by plants (Edelstein and Ben-Hur 2018). The most important factors affecting the absorption of HMs from the soil are soil pH, metal accessibility, geographical location, agricultural factors such as different fertilizers, interaction with other elements and genotypes (Oliveira et al. 2022). The roots of plants secrete a variety of organic acids such as oxalate, aspartate, citrate, and malate (as ligand) to the soil, which changes the solubility of metals in the soil that are in the form of insoluble (Sharma et al. 2020).

HMs are effective on plant growth and development, seed germination and seedling growth, water relations, nutritional status, cellular organelles, ions, organic compounds especially proteins (enzymes) and nucleic acids, production of reactive oxygen species (ROSs) and homeostasis and can causes senescence and cell programmable death (Godinho et al. 2018; Sharma et al. 2020).

Metals (including HMs) alter water relations in plants. HMs reduce water transport from roots to shoots leading to a severe dehydration of shoots (Yusuf et al. 2011). For example, Ni reduced plant transpiration rate and water content in *Brassica oleracea* and *Phaseolus vulgaris* (Molas 1997). Reduction or prevention from photosynthesis and respiration occurs by the effect of HMs on most components of photosynthetic and respiratory apparatus, including pigments and enzymes (Abbas et al. 2018). HMs increase the activity of chlorophyllase enzyme and move central magnesium ion (Mg^{2+}) in the porphyrin ring (Riyazuddin et al. 2021). The decrease in the number of soil microorganisms due to the high concentration of HMs can affect root growth and indirectly to the entire plant (Asati et al. 2016).

Plants respond to HMs in different ways such as the use of enzymatic and non-enzymatic antioxidant systems. Restricting the absorption of metals or limiting their transmission to the shoot system, storing them in cell wall, inter-cellular spaces, Golgi apparatus or vacuole together with the formation of HMs complex with ligands, production or accumulation of solutions or adaptive osmolytes and changing metal status (conversion of their status and connection to the ligands) are other approaches to de-toxicity (Kabata-Pendias 2011; Abbas et al. 2018). Reduction of the leaf thickness and change in its inter-cellular spaces are of the anatomical properties of *Taraxacum officinale* Web growing in the soil with high concentrations of Cd, Cr, Cu, Fe, Pb and Zn (Bini et al. 2012). Excessive of Cr usually restricts the absorption of nutrients by formation of insoluble compounds, makes a negative effect on chlorophyll levels and photosynthesis, changes indigenous levels of hormones, and produces ROSs which causes oxidative stress. Subsequently, ROSs cause damage to DNA, lipids, pigments and proteins and stimulate the lipid peroxidation (LPO) phase with increased malondialdehyde (MDA) (Kabata-Pendias 2011; Sharma et al. 2020). The high concentration of Cr decreased growth and biomass of some plants including *Albizia lebbek*, *Acacia holosericea*, *Vallisneria spiralis*, *Portulaca oleracea* and *Leucaena leucocephala* (Shanker et al. 2005). Study on *Pyrus* spp. and *Cydonia oblonga in vitro* showed that the total chlorophyll of the micro-shoots was reduced in all studied genotypes under Fe-deficit conditions (Şahin et al. 2022). Both low or high Fe concentrations reduced the growth and accumulation of biomass in cucumbers in hydroponic conditions, induced chlorosis and oxidative stress, and reduced chlorophyll content and photosynthesis and respiration in the leaves (Ahammed et al. 2020). The accumulation of some metabolites such as organic acids was increased in some tolerated plants such as *Malus halliana* to Fe deficiency (Zhang et al. 2019). Excessive amount of Co changes plant biomass, limit Fe concentration and effect on phosphorus (P), sulfur (S), Mn, Zn and Cu, reduces water potential, transpiration rate, shoot and root growth, leaf surface, and nutrients including sugar, and affect chlorophyll, amino acid, protein and activity of some enzymes, including antioxidant enzymes (Asati et al. 2016). Co plays an important role in the production of ethylene in plants (Akeel and Jahan 2020). High levels of Co leads to chlorosis and necrosis, prevents root formation, and disturb nutrient and water absorption (Akeel and Jahan 2020).

Ni toxicity reduced growth, leaf water content, chlorophyll content, inter-cellular carbon dioxide (CO_2) concentrations, stomatal conductivity and transpiration rate and increased hydrogen peroxide (H_2O_2), MDA and ionic leakage in *Solanum melongena* L. (Ali Shah et al. 2021). Exogenous application of Ni can improve the biosynthesis of amino acids, nitrogen metabolism and respiratory cycles in tomatoes under nitrogen deficiency conditions (Li et al. 2022). The use of high concentrations of Zn and Cu reduced the production of biomass and growth of *Phytolacca americana* and showed symptoms of toxicity related to the reduction of antioxidant enzymes activity (Zhao et al. 2012). Cu accumulation in the soil causes the effect on soil quality and its microbial variety (Leguina et al. 2019). Zn is a vital cofactor for some important enzymes, but its high concentration can lead to numerous changes such as growth, photosynthesis and respiration, uneven mineral nutrition, enzymes activity, and increased ROS production (Kaur and Garg 2021; Natasha et al. 2022). As is one of the most toxic metals for living organisms. As intersects with –SH groups of proteins and enzymes, preventing cellular function and ultimately cell death (Armendariz et al. 2016; Pandey et al. 2017). Treatment of *Cicer arietinum* with As reduced the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Al-Huqail et al. 2017). In *Pisum sativum*, As changed the dry weight of the root and shoot, damaged chlorophyll pigments and induced oxidative stress by reducing membrane stability and increased H_2O_2 production (Garg and Singla 2012).

Among HMs, Cd is one of the most toxic elements for the plants due to its high stability and mobility (Abu-Shahba et al. 2022). Biological function of Cd in plants and animals is not well known. Cd is the third pollutant and the biggest risk for environment after mercury (Hg) and Pb and is the only metal that is harmful to human and animals' health at concentrations that are not usually toxic in plant tissues (Ismael et al. 2019). Under Cd stress, high production of ROS can cause physiological irregularities in plants that lead to decrease in growth and biomass and metabolic hemostasis disruption (Mahmoud et al. 2021). The study on cocoa showed that the tree proceeded some strategies such as absorption and transfer of Cd from root to shoot, molecular and biochemical changes, compartmentation between organs and sequestration at the cellular surface to reduce the Cd toxicity (Oliveira et al. 2022).

The concentration of more than 20 mg/l Cd increased the death of 9 orange stocks, although at a concentration of 100 mg/l all the stocks were destroyed (Chun et al. 2020). Cd stress significantly reduced the activity of CAT, SOD, APX, glutathione synthesis enzyme, also biomass and efficiency in strawberry, but improved ascorbate oxidase activity, glutathione reductase, ascorbate-synthesized enzyme and the content of MDA and H_2O_2 (Zhang et al. 2020). The study on *Virola surinamensis*, a forest tree, showed that Cd reduced the potential of leaf water, stomatal conductivity and transpiration. With the increase in Cd concentration, the maximum photochemical efficiency of photosystem II (PS-II), the amount of electron transmission, the photochemical quiescence coefficient, and the total chlorophyll rate declined due to the decrease in photosynthesis (Júnior et al. 2019). In chicory, root and shoot growth and biomass accumulation decreased during stress with Pb and aluminum (Al). The oxidative stress indicators such as MDA, H_2O_2 and osmolytes were significantly increased with metal treatments. The antioxidant enzyme defense system was positively associated with increased Pb and Al stress (Malik et al. 2021). The combined toxicity of HMs is usually higher than their single toxicity (Kaya et al. 2019). Increased combination of both Pb and Cd to peppermint nutritional solution caused an effect on dry weight, PS-II efficiency, chlorophyll content, leaf water content, ionic leakage, H_2O_2, MDA and proline, which in turn increased the activities of key leaf enzymatic antioxidants including peroxidase (POD), SOD, CAT and lipoxygenase and non-enzymatic antioxidants levels of ascorbate and glutathione (Kaya et al. 2019). *Impatiens balsamina*, *Mirabilis jalapa*, and *Tagetes erecta* exhibited strong tolerance to Sn contamination, and visual toxicity was not observed for these three plants grown under highest the Sn treatments. The content of Sn accumulated in these three plants was positively correlated with the Sn concentration in the soil (Liu et al. 2021).

Urban horticulture is expanding in most cities around the world due to overpopulation, traffic, pollution and industrial towns, and in the near future, the quality of life will become an essential feature of urban design (Delaman and Farahmand 2017). Accumulated-HMs grass and wooden plants (trees and shrubs) are used for phytoremediation. To develop improved approaches to enhance phytoremediation efficiency, the knowledge of micro-structural, physiological and molecular responses that accumulate HMs is needed. These responses are mainly related to the absorption, transmission, sequestration and de-toxicity, as well as adjusting these steps by the transmission of signal in response to HMs (Luo et al. 2016). Literature data revealed that extraction, absorption, transmission, and accumulation of HMs, also resistance, tolerance, hardening, and other strategic responses to HMs is plant species- and even organ-dependent and varies with specific metal, concentration, chemical form and soil composition and pH (Asati et al. 2016). The purpose of the present investigation was to measure the concentration of 10 HMs (As, Cd, Cr, Co, Cu, Fe, Pb, Ni, Sn, and Zn) in 10 dominant ornamental species (*Ficus religiosa*, *Ficus elastica*, *Syzygium cumini*, *Azadirachta indica*, *Clerodendrom inerme*, *Conocarpus erectus*, *Bougainvillea* sp., *Delonix regia*, *Dodonaea viscosa*, and *Phoenix* sp.) grown in Bushehr, Iran, and their influence on some physiological parameters and antioxidant enzymes activity for the expansion of green spaces.

Materials And Methods

Study site

The city of Bushehr, capital of Bushehr Province in southern part of Iran, located in the south and west of the Persian Gulf, is a port town (Lat. 46°29' N., Long. 40°51' E., Alt. 8 m.) (Fig. 1). Bushehr has a hot semi-arid climate with a precipitation pattern resembling a Mediterranean climate, albeit it is both too hot and dry for too long to qualify as such, by a wide margin, due to the threshold for hot climates being much higher in terms of required rainfall. Its precipitation rate is 268 mm, the number of rainy days is 22, and the average relative humidity is 65%. The maximum temperature occurring in July-August is 44.8°C and the minimum occurring in January-February is 5°C (Meteorological statistics of Bushehr based on the data of Meteorological Organization of Bushehr Province for 1991–2021). The highest and lowest recorded temperatures are 50 and − 1°C, respectively. The average annual temperature of Bushehr is 25°C. Bushehr is almost the same sea level, although in some parts it is lower than sea level. The city is only on the east side to land. The city of Bushehr does not have significant vegetation due to its position, poor soil and salinity. Bushehr soil has pH 7.8 and EC 8.89 ds/m and its texture has a higher clay percentage with moderate permeability. Bushehr has hot and humid climate due to its lower geographical latitude, proximity to Apt orbit, a little height compared to sea level, low vegetation, location on the hot winds of Saudi Arabia, and a little participation. The city of Bushehr has a population of around 323,000 located in the southern part of Bushehr Province. It has 69 parks and its per capita green space is about 6.89 m^2 (Anonymous, 2017). Three regions were selected as the study sites (Fig. 1).

Selection Of Plant Species

There is no established standard for plant species selection criteria. For this reason, for the selection of the suitable plant species, the list of all ornamental plants available in the Bushehr City Parks and Green Space Organization was prepared. This list contained 123 species of ornamental plants with bush, shrub and tree phenology. Of these 123 plant species, the top 10 species (Fig. 2) were selected based on some indices such as growth parameters, regional adaptation, urban environment, maintenance, aesthetics, and specific advantages in the city (Asgharpour 2009). It was consulted with some organizations, including the Parks and Urban Green Space Organization, the Environmental Organization, and experts in the field of horticulture, green space and urban architecture. The list of the top 10 species of ornamental plants, along with some of most important their features is listed in Table 1.

Table 1
Selected ornamental plants, their family, phenology and some other important characteristics

Plant species	Family	Phenology	Some other characters
Ficus religiosa	Moraceae	Shrub	Semi-evergreen or deciduous, dicot, thermophile
Ficus elastica	Moraceae	Shrub	Evergreen, dicot, resistance against water deficit
Syzygium cumini	Myrtaceae	Tree	Evergreen, biological tree
Azadirachta indica	Meliaceae	Shrub	Deciduous, dicot, resistance against heat
Clerodendrom inerme	Lamiaceae	Shrub	Evergreen or deciduous, dicot, resistance against various stresses such as salinity
Conocarpus erectus	Combretaceae	Tree	Evergreen, coniferous, suitable for air conditioning
Bougainvillea sp.	Nyctaginaceae	Shrub	Evergreen or deciduous, dicot, resistance against pollutions
Delonix regia	Fabaceae	Tree	Evergreen or deciduous, dicot, resistance against water deficit
Dodonaea viscosa	Sapindaceae	Shrub	Evergreen, resistance against heat
Phoenix sp.	Arecaceae	Tree	Evergreen, monocot, resistance against water deficit and heat

Preparation Of Leaf Specimens

Three locations were selected for sampling at the city of Bushehr (Fig. 1). In each location, 4 trees and shrubs were considered out of every 10 ornamental plants and about 30 leaves were separated. In total, about 80 to 100 g of leaf sample was harvested. The samples were transferred to a laboratory in the city of Bushehr to measure the amount of heavy metals. To measure physiological traits and enzymatic activity, the samples were vacuum in plastic containers and sent to Rasht city.

Selection And Measurement Of Hms

Ten HMs, including arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), tin (Sn), and zinc (Zn) were considered to measure their concentration in leaf tissues of 10 selected plants. The content of 5 g of each plant sample was placed at 500°C for 8 h until powdered and converted to ashes. To measure the concentration of HMs in the selected plants, 0.2 g of powdered leaf sample (ashes) was placed in 3 ml nitric acid 65% for 24 h at room temperature. The solution was then placed on the asbestos heater at 110°C for 5 h until acidic digestion. The resulting solution was filtered in a 10 mm balloon with Whatman 42 filter paper and was volume with nitric acid 1% or with water and was injected into the inductively coupled plasma mass spectrometry (ICP-MS) instrument (a type of mass spectrometry) to measure the concentration of HMs. The material reference was used to calibrate the numbers and the concentration of HMs was read in mg per liter.

Measurement Of Physiological Parameters

Relative leaf water

Leaves specimens were separated from plants and their fresh weight (F.W.) was measured immediately with a digital scale to the nearest 0.01 g. After measurement of F.W., the leaves were dried in an oven at 105°C for 24 h and their dry weight (D.W.) was measured with a digital scale to the nearest 0.01 g. Percentage of relative leaf water was calculated with following equation.

$$\text{Relative leaf water } (\backslash\%) = \frac{\text{Fresh weight}}{\text{Dry weight}} \times 100$$

Dry Matter

To measure the D.W. of the leaves, they were dried for 24 h at an oven at 105°C and their weight was obtained with a digital scales of 0.05 g. Dry matter percentage was calculated with following equation.

$$\text{Dry matter } (\backslash\%) = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Vitamin C (Ascorbic Acid)

The vitamin C ($C_6H_8O_6$) content was measured using the titration method with 2,6-dichlorophenol-indophenol (DCIP) for which 2 g of the leaves was extracted with 20 ml of 3% metaphosphoric acid (HPO_3) and was mixed in a homogenizer for 30 min. The extract was filtered through a Whatman paper. At the next step, 15 ml of the filtered extract was titrated with DCIP until the emergence of pink color at which step the amount of DCIP applied for titration was recorded. Finally, the following equation was applied to determine the vitamin C content of the leaves in mg 100 g^{-1} F.W. (Mazumdar and Majumdar 2003).

$$\text{Vitamin C } (\backslash\%) = \frac{\text{e} \times \text{d} \times \text{b}}{\text{c} \times \text{a}} \times 100$$

In which; “a” is the sample weight, “b” is the volume of metaphosphoric acid used for extraction, “c” is the volume of the solution taken for titration, “d” is the color factor, and “e” is the volume of the colored solution consumed.

Acidity (Ph)

To measure the acidity of plant sample leaf, 50 g of each sample was well grinded inside the Chinese mortar with 100 mg/l of distilled water in three steps. The resulting solution was filtered with Whatman filter paper No. 42. The acidity of the plant samples was measured by the pH-meter. Tampon solutions with acidities 4 and 7 were used to adjust the device.

Chlorophylls

To measure chlorophyll a, b, and total chlorophyll, the leaves were sampled and extracted in 80% acetone (C_3H_6O). The absorption of the filtered samples was read at 643 and 660 nm with an APEL PD-330 UV spectrophotometer, and chlorophyll a, b, and total chlorophyll were calculated by the following equations (Mazumdar and Majumdar, 2003).

$$\text{Chlorophylla} = 9.93\,(\text{A}_{660}) - 0.777\,(A_{643})$$

$$\text{Chlorophyllb} = 17.60\,(\text{A}_{643}) - 2.81\,(A_{660})$$

$$\text{Totalchlorophyll} = 7.12\,(\text{A}_{660}) + 16.8\,(A_{642})$$

Carotenoid

To measure leaves carotenoids, extraction was performed with 80% acetone. The absorbance of the extract was read with a spectrophotometer (Shimadzu UV-120-02, Japan) at 440, 645, and 663 nm and then, the following equation was used to calculate carotenoid content in μg g^{-1} F.W. (Mazumdar and Majumder 2003).

$$\text{Petalcarotenoids} = (4.69 \times A_{440}) - (0.286 \times 20.2 \times A_{645}) + (8.02 \times A_{663})$$

Anthocyanin

Acidic methanol ($C_3H_8O_3$) (pure methanol + 1% HCl) was used to extract the anthocyanin from leaves. The absorbance of the infiltrated extract was read at 535 nm with a spectrophotometer (Shimadzu UV-120-02, Japan), and anthocyanin content was calculated by the following equation (Mazumdar and Majumder 2003).

$$\text{Anthocyanin (mg/100 g F.W.)} = \frac{e \times b \times c}{d \times a} \times 100$$

In which; “e” is the sample weight, “b” is the volume of the sample taken for the measurement, “c” is the total prepared solution, “d” is the volume of the taken sample, and “a” is the reading.

Total Flavonoid

To measure the content of total flavonoid, 0.1 g of the leaves were extracted with 2.5 ml of acidic ethanol (99% ethanol + 1% glacial acetic acid) and centrifuged at 3600 rpm for 15 min. The resulting supernatant was extracted with a sampler and was placed in a hot-water bath at 85°C for 10 min. The absorption of the samples was then read at 330 nm with a PD-3000 UV APEL spectrophotometer (Mazumdar and Majumder, 2003).

Total Protein

Total protein content of leaves was measured by Kjeldahl’s indirect method. So, first nitrogen percentage and then protein percentage were calculated by the following equation.

$$\text{Nitrogen (\%)} = 0.56 \times t \times (a - b) \times \frac{v}{w} \times \frac{100}{DM}$$

In which; “t” is the concentration of acid used for titration in mol l^{-1}, “a” is the amount of acid used for sample in mol l^{-1}, “b” is the amount of acid used as control in ml, “v” is the volume of extract taken from digestion in ml, “w” is the plant sample weight for digestion in g, and “DM” is the plant D.W.

Total Soluble Carbohydrates

Anthrone reagent ($C_{14}H_{10}O$) was used to measure the total carbohydrate content. To prepare this reagent, 200 mg of anthrone was dissolved in 100 ml of sulfuric acid (H_2SO_4) 95% (glacial). The 100 mg of leaf of each plant species was weighed and put in boiling test tube. The test tube was placed in the boiling water bath for 3 h by adding 5 ml of hydrochloric acid (HCl) 2.5 N for hydrolysis of the above solution and then cooled at room temperature. The solution was neutralized with solid sodium carbonate (Na_2CO_3) until the boiling was stopped. The solution volume was reached to 100 ml and centrifuged. The supernatant was collected and 0.5-1.0 ml was used for analysis. Carbohydrate standard was prepared. The solution volume was increased to 1 ml in all test tubes by distilled water and 4 ml of anthrone reagent was added to each tube. The tubes were heated in the boiling water bath for 8 min. After cooling the solutions, their absorption was read at 630 nm by a spectrophotometer. The standard curve was plotted by the standard concentration position on the axis “X” and the absorption value on the axis “Y”, and the amount of carbohydrates in each sample was calculated with the following equation.

$$\text{Hydrocarbon amount in 100 ml of leaf (\%)} = \frac{\text{Glucose (mg)}}{\text{Test tube volume}} \times 100$$

Proline

Proline content was determined spectrophotometrically by adopting the ninhydrin (2,2-dihydroxyindane-1,3-dione) method of Bates et al. (1973). The 300 mg of fresh leaf sample was homogenized in 3 ml of 3% sulfosalicylic acid ($C_7H_6O_6S$). The homogenate filtrate was reacted with 1 ml each of ninhydrin and glacial acetic acid (CH_3COOH) for 1 h in a test tube placed in a water bath at 100°C. The mixture was extracted with toluene and the absorbance was measured on a spectrophotometer (JASCO Model V-530) at 520 nm using L-proline as a standard.

Measurement Of Antioxidant Enzymes Activity

Peroxidase (POD)

To measure the activity of antioxidant enzymes, an enzymatic extract was first prepared for which the leaves of 10 ornamental plants were extracted with 50 mmol potassium phosphate (K_3PO_4) buffer. Then, it was centrifuged at 10,500 rpm at 4°C for 25 min. Using a sampler, the transparent supernatant of the sample was taken and used as the enzymatic extract. POD activity was measured by In et al. (2007) method for which some leaves samples were detached and was extracted with 50 mM of K_3PO_4 buffer. The extract was centrifuged at 10,500 rpm at 4°C for 25 minutes. The supernatant was used as the enzymatic extract. So, 100 µl of the extract was added with 450 µl of H_2O_2 and 450 µl of guaiacol ($C_7H_8O_2$) solution. Then, it was read at 470 nm with a JASCO Model V-530 spectrophotometer and POD activity was reported in nmol g^{-1} F.W. min^{-1}.

Superoxide Dismutase (Sod)

To estimate SOD activity, 0.1 ml of the enzymatic extract was mixed with 25 ml of nitro blue tetrazolium chloride (NBT) ($C_{40}H_{30}C_{l2}N_{10}O_6$), 0.1 mmol of ethylenediaminetetraacetic acid (EDTA) ($C_{10}H_{16}N_2O_8$), 13 mmol of methionine ($C_5H_{11}NO_2S$), 50 mmol of K_3PO_4 buffer, and 50 mmol of Na_2CO_3 and was placed in a shaker at 22°C under fluorescence to be shaken gently for 30 min. At the next step, the samples were kept in a dark room for 30 min. Then, the absorbance was read at 560 nm with a spectrophotometer (JASCO V530, Japan) and reported in IU g^{-1} F.W. min^{-1} (Giannopolitis and Ries 1997).

Experimental Design And Data Analysis

The experiments were established in a completely randomized block design with three replicates. Data were subjected to analysis of variance (ANOVA) and means were compared by the LSD test at $P < 0.05$ using the SAS ver. 9.2 software.

Results

HMs contents

According to the analysis of variance (Table 2), differences between selected plant species related to the accumulation of HMs in their leaves tissues was significant ($P < 0.05$ for Fe and $P < 0.01$ for other HMs). Among selected HMs; Cu, Fe, Ni, and Zn are essential elements (micronutrients), Co is beneficial element, and As, Cd, Cr, Pb, and Sn are non-essential elements.

Table 2
Analysis of variance of 10 heavy metals contents in selected ornamental plants

Source of variance	df	Cr	Fe	Co	Ni	Cu	Zn	As	Sn	Pb
Repetition	2	3.79^{ns}	7674.00^{ns}	0.0438^{ns}	0.146^{ns}	7.48^{ns}	148.70^{ns}	0.00000009^{ns}	0.037^{ns}	0.0765^{ns}
Treatment	9	19.26^{**}	26091.00^{*}	0.1622^{**}	7.37^{**}	331.30^{**}	656.10^{**}	0.0000169^{**}	0.125^{**}	1.8036^{**}
Error	18	5.21	7754.00	0.0138	0.1237	8.075	92.80	0.00000018	0.0249	0.119
CV (%)	-	22.85	34.77	35.09	10.01	13.59	24.20	8.43	6.94	31.11

* and **: Significant at the 5 and 1% probability levels, respectively. ^{ns}: Non-significant

C. inerme had greatest amount of As (0.0087 mg/kg dry weight (D.W.), and Sn (2.516 mg/kg D.W.) than other species. This species was rich in Ni, Co and Zn (Table 3). The results showed that the levels of Zn in *D. viscosa*, *C. inerme*, and *C. erectus* was high and ranged between 50.73–55.73 mg/kg D.W. Highest content of Cr (14.71 mg/kg D.W.), Cu (45.52 mg/kg D.W.), and Zn (61.86 mg/kg D.W.) was obtained in *Bougainvillea* sp. The content of Cr and Cu in *Bougainvillea* sp. was much more than other ornamental plants. Within the selected ornamental plants, the highest concentration of Co (0.686 mg/kg D.W.) was noticed in *C. erectus* followed by *D. viscosa* (0.655 mg/kg D.W.). Results also showed that the maximum amount of Fe (428.00 mg/kg D.W.), and Ni (6.448 mg/kg D.W.) was extracted from *C. erectus*, while the amount of Sn in this species was minimum. The lowest rate of Fe (155 mg/kg D.W.) and Sn (1.836 mg/kg D.W.) was observed in *F. religiosa*. Also, the content of Cr, Co, and Zn was low in this ornamental species, while the content of As was high in its leaf tissues (Table 3). Fe concentration in leaves of *C. erectus* was about two or three times over than that of in other selected ornamentals.

Table 3
Mean comparison of the contents of 10 heavy metals in 10 selected ornamental plants

Plant species	Arsenic (As)	Cadmium (Cd)	Chromium (Cr)	Cobalt (Co)	Copper (Cu)
Ficus religiosa	$0.0076^{b} \pm 0.0005$	< 0.001	$7.07^{d} \pm 0.86$	$0.153^{de} \pm 0.02$	$18.24^{cd} \pm 0.23$
Ficus elastica	$0.0060^{c} \pm 0.0000$	< 0.001	$11.34^{a-c} \pm 0.55$	$0.211^{c-e} \pm 0.02$	$32.27^{b} \pm 1.78$
Syzygium cumini	$0.0050^{d} \pm 0.0000$	< 0.001	$8.72^{cd} \pm 0.60$	$0.122^{e} \pm 0.04$	$15.89^{c-e} \pm 4.71$
Azadirachta indica	$0.0072^{b} \pm 0.0002$	< 0.001	$13.18^{ab} \pm 5.34$	$0.351^{cd} \pm 0.14$	$13.46^{de} \pm 0.99$
Clerodendrom inerme	$0.0087^{a} \pm 0.0000$	< 0.001	$8.46^{cd} \pm 3.50$	$0.569^{ab} \pm 0.20$	$20.70^{c} \pm 0.10$
Conocarpus erectus	$0.0036^{e} \pm 0.0005$	< 0.001	$11.24^{a-c} \pm 0.55$	$0.686^{a} \pm 0.19$	$20.07^{c} \pm 0.76$
Bougainvillea sp.	$0.0043^{de} \pm 0.0005$	< 0.001	$14.71^{a} \pm 0.33$	$0.401^{bc} \pm 0.10$	$45.52^{a} \pm 4.71$
Delonix regia	$0.0040^{e} \pm 0.0000$	< 0.001	$7.96^{cd} \pm 0.54$	$0.119^{e} \pm 0.02$	$19.43^{c} \pm 5.21$
Dodonaea viscosa	$0.0013^{g} \pm 0.0005$	< 0.001	$7.75^{cd} \pm 1.47$	$0.655^{a} \pm 0.22$	$11.38^{e} \pm 1.89$
Phoenix sp.	$0.0023^{f} \pm 0.0005$	< 0.001	$9.40^{b-d} \pm 2.36$	$0.089^{e} \pm 0.03$	$12.11^{e} \pm 0.28$

Means with different letters on the same column are significantly different ($p < 0.05$) based on LSD test. Units are in mg/kg D.W.

Table 3
Continued

Plant species	Iron (Fe)	Lead (Pb)	Nickel (Ni)	Tin (Sn)	Zinc (Zn)
Ficus religiosa	$155.00^{c} \pm 4.25$	$0.818^{d} \pm 0.123$	$2.231^{f} \pm 0.14$	$1.836^{b} \pm 0.047$	$29.33^{cd} \pm 3.60$
Ficus elastica	$241.00^{bc} \pm 13.29$	$2.499^{a} \pm 0.390$	$3.631^{d} \pm 0.16$	$2.413^{a} \pm 0.050$	$29.42^{cd} \pm 2.83$
Syzygium cumini	$209.00^{bc} \pm 6.36$	$2.093^{ab} \pm 0.103$	$4.033^{cd} \pm 0.49$	$2.396^{a} \pm 0.126$	$25.52^{cd} \pm 0.98$
Azadirachta indica	$269.00^{bc} \pm 5.20$	$0.176^{ef} \pm 0.018$	$4.603^{c} \pm 0.34$	$2.366^{a} \pm 0.100$	$22.85^{d} \pm 0.63$
Clerodendrom inerme	$227.00^{bc} \pm 7.48$	$1.139^{c} \pm 0.548$	$5.267^{b} \pm 0.40$	$2.516^{a} \pm 0.083$	$54.78^{ab} \pm 4.45$
Conocarpus erectus	$428.00^{a} \pm 276.23$	$1.087^{cd} \pm 0.508$	$6.448^{a} \pm 0.65$	$1.986^{b} \pm 0.011$	$55.73^{ab} \pm 5.61$
Bougainvillea sp.	$222.00^{bc} \pm 8.02$	$1.075^{cd} \pm 0.508$	$2.959^{e} \pm 0.23$	$2.312^{a} \pm 0.410$	$61.86^{a} \pm 9.20$
Delonix regia	$311.00^{ab} \pm 27.36$	$0.679^{de} \pm 0.027$	$1.845^{f} \pm 0.35$	$2.310^{a} \pm 0.110$	$41.05^{bc} \pm 18.39$
Dodonaea viscosa	$232.00^{bc} \pm 3.15$	$0.087^{f} \pm 0.009$	$2.053^{f} \pm 0.07$	$2.350^{a} \pm 0.173$	$50.73^{ab} \pm 22.05$
Phoenix sp.	$234.00^{bc} \pm 3.78$	$1.546^{bc} \pm 0.310$	$2.070^{f} \pm 0.23$	$2.316^{a} \pm 0.115$	$26.62^{cd} \pm 1.23$

Means with different letters on the same column are significantly different (p < 0.05) based on LSD test. Units are in mg/kg D.W.

The highest amount of Pb (2.499 mg/kg D.W.) was calculated in *F. elastica* followed by *S. cumini*. *F. elastica* was rich in Sn. Least concentration of As (0.0013 mg/kg D.W.), Cr (7.75 mg/kg D.W.), Cu (11.38 mg/kg D.W.), and Pb (0.087 mg/kg D.W.) was detected in *D. viscosa*. *Phoenix* sp. was a species with minimum Co (0.089 mg/kg D.W.), and low As (0.0023 mg/kg D.W.), Cu (12.11 mg/kg D.W.), and Zn (26.62 mg/kg D.W.). Also, *D. regia* had minimum Ni (1.845 mg/kg D.W.), and low Cr (7.96 mg/kg D.W.), and Co (0.119 mg/kg D.W.). The lowest content of Zn (22.85 mg/kg D.W.), and low content of Pb (0.176 mg/kg D.W.), and Cu (13.46 mg/kg D.W.) was related to *A. indica* (Table 3). Differences between concentration of Cd in all 10 selected ornamental plants were not significant (Table 3).

Physiological Parameters And Antioxidant Enzymes Activity

HMs induced physiological changes in selected species. Based on the results of analysis of variance (Table 4), differences between selected plant species related to the changes in measured physiological characteristics and antioxidant enzymes activity in their tissues was significant ($P < 0.01$).

Table 4
Analysis of variance of the effect of heavy metals on physiological responses and antioxidant enzymes activity in selected ornamental plants

Source of variance	df	Relative content of leaf water	Dry matter percentage	Vitamin C content	pH	Chlorophyll a content	Chlorophyll b content	Total chlorophyll content	Carotenoid content
Repetition	2	0.00380^{ns}	0.289^{ns}	0.234^{ns}	0.00756^{ns}	0.864^{ns}	0.0608^{ns}	1.823^{ns}	0.933^{ns}
Treatment	9	34.45^{**}	132.5^{**}	5.48^{**}	0.7443^{**}	17.59^{**}	5.522^{**}	35.27^{**}	32.64^{**}
Error	18	1.048	0.905	0.465	0.034	0.539	0.102	1.066	0.695
CV (%)	-	1.25	2.91	10.05	3.03	6.93	6.02	6.51	5.67

* and **: Significant at the 5 and 1% probability levels, respectively. ^{ns}: Non-significant

Table 4
Continued

Source of variance	df	Anthocyanin content	Total flavonoid content	Total protein content	Total soluble carbohydrates	Proline content	POD activity	SOD activity
Repetition	2	0.0970^{ns}	0.074^{ns}	0.0011^{ns}	2.209^{ns}	0.00056^{ns}	0.0491^{ns}	33.13*
Treatment	9	32.928**	1.0927**	0.515**	126.3**	0.3429**	0.879**	187.0**
Error	18	1.464	0.034	0.0041	0.7913	0.00311	0.01788	6.12
CV (%)	-	8.35	7.05	4.22	9.74	10.39	23.83	4.17
* and **: Significant at the 5 and 1% probability levels, respectively. ^{ns}: Non-significant								

Phoenix sp. had maximum relative rate of leaf water (88.95%) and dry matter percentage (46.03). This ornamental species also had low chlorophyll a, chlorophyll b, and total protein (Table 5). The amount of relative leaf water in *F. elastica*, *S. cumini*, *A. indica*, *C. erectus*, *D. regia*, and *D. viscosa* was high and ranged between 81.64–83.12%. The lowest amount of relative leaf water (around 77.50%) was obtained in *F. religiosa*, *C. inerme*, and *Bougainvillea* sp. *C. inerme* with 21.10% had least dry matter percentage. This ornamental species also showed the lowest content of chlorophyll a (7.04 µg/ml), total chlorophyll (11.10 µg/ml), and carotenoid (10.22 µg/g F.W.), and low content of anthocyanin (11.38 mg/100 g F.W.). Highest value of pH (6.905), and the highest amount of chlorophyll a (14.61 µg/ml), total chlorophyll (21.86 µg/ml), carotenoid (20.83 µg/g F.W.), and anthocyanin (18.81 mg/100 g F.W.) was obtained in *F. elastica*. Chlorophyll b content was high in this species. *F. religiosa* showed high amount of chlorophyll a (13.95 µg/ml), total chlorophyll (19.90 µg/ml), carotenoid (18.57 µg/g F.W.), and anthocyanin (18.55 mg/100 g F.W.) in its leaf tissues (Table 5).

Table 5
Mean comparison of the effect of heavy metals on physiological responses and antioxidant enzymes activity in selected ornamental plants

Type of plants	Relative rate of leaf water (%)	Dry matter (%)	Vitamin C content (mg/100 g F.W.)	pH	Chlorophyll a content (µg/ml)	Chlorophyll b content (µg/ml)	Total chlorophyll content (µg/ml)	Carotenoid content (µg g⁻¹ F.W.)
Ficus religiosa	$77.08^{c} \pm 1.34$	$33.70^{c} \pm 1.11$	$5.78^{de} \pm 0.34$	$6.640^{ab} \pm 0.13$	$13.95^{a} \pm 1.16$	$5.96^{b} \pm 0.19$	$19.90^{b} \pm 1.36$	$18.57^{b} \pm 0.65$
Ficus elastica	$82.64^{b} \pm 0.99$	$33.54^{c} \pm 0.75$	$4.93^{ef} \pm 0.51$	$6.905^{a} \pm 0.13$	$14.61^{a} \pm 0.78$	$7.26^{a} \pm 0.57$	$21.86^{a} \pm 1.36$	$20.83^{a} \pm 1.57$
Syzygium cumini	$81.78^{b} \pm 0.35$	$38.40^{b} \pm 1.51$	$8.50^{a} \pm 1.02$	$5.765^{e} \pm 0.14$	$10.06^{cd} \pm 0.56$	$7.63^{a} \pm 0.30$	$17.69^{c} \pm 0.87$	$13.34^{d} \pm 0.90$
Azadirachta indica	$83.12^{b} \pm 1.47$	$32.85^{c} \pm 0.65$	$7.48^{a-c} \pm 0.68$	$6.160^{cd} \pm 0.06$	$8.88^{de} \pm 0.09$	$5.22^{cd} \pm 0.06$	$14.10^{d} \pm 0.15$	$13.02^{de} \pm 0.47$
Clerodendrom inerme	$77.55^{c} \pm 0.99$	$21.10^{f} \pm 0.44$	$7.99^{ab} \pm 0.17$	$5.400^{f} \pm 0.15$	$7.04^{f} \pm 0.14$	$4.07^{e} \pm 0.19$	$11.10^{e} \pm 0.05$	$10.22^{f} \pm 0.28$
Conocarpus erectus	$81.79^{b} \pm 0.85$	$30.35^{d} \pm 0.98$	$6.63^{cd} \pm 0.51$	$5.365^{f} \pm 0.39$	$8.80^{e} \pm 0.28$	$4.89^{d} \pm 0.17$	$13.68^{d} \pm 0.46$	$11.84^{e} \pm 0.23$
Bougainvillea sp.	$78.65^{c} \pm 0.72$	$29.10^{d} \pm 0.65$	$6.46^{cd} \pm 0.34$	$5.875^{de} \pm 0.03$	$10.24^{c} \pm 0.63$	$3.54^{e} \pm 0.02$	$13.28^{d} \pm 1.16$	$12.74^{de} \pm 0.37$
Delonix regia	$82.23^{b} \pm 0.89$	$27.22^{e} \pm 0.72$	$4.59^{f} \pm 0.51$	$6.420^{bc} \pm 0.25$	$11.86^{b} \pm 0.33$	$5.59^{bc} \pm 0.16$	$17.45^{c} \pm 0.49$	$16.96^{c} \pm 0.12$
Dodonaea viscosa	$81.64^{b} \pm 1.05$	$34.42^{c} \pm 0.75$	$8.16^{ab} \pm 0.68$	$6.050^{de} \pm 0.10$	$11.74^{b} \pm 1.14$	$4.93^{d} \pm 0.63$	$16.67^{c} \pm 1.77$	$16.04^{c} \pm 1.24$
Phoenix sp.	$88.95^{a} \pm 0.45$	$46.03^{a} \pm 1.11$	$7.31^{bc} \pm 1.19$	$6.125^{cd} \pm 0.01$	$8.80^{e} \pm 1.22$	$3.94^{e} \pm 0.18$	$12.74^{de} \pm 1.40$	$13.38^{d} \pm 1.19$

Means with different letters on the same column are significantly different ($p < 0.05$) based on LSD test

Table 5

Continued

Type of plants	Anthocyanin content (mg/100 g F.W.)	Total flavonoid content (M/g)	Total protein content (%)	Total soluble carbohydrates (%)	Proline content (µM/g F.W.)	POD activity (U/g F.W./min)	SOD activity (U/g F.W.)
Ficus religiosa	18.55^{a} ± 0.17	2.36^{c} ± 0.03	1.21^{e} ± 0.09	7.63^{cd} ± 0.71	0.39^{ef} ± 0.013	0.612^{c} ± 0.012	66.35^{ab} ± 5.88
Ficus elastica	18.81^{a} ± 0.73	2.33^{c} ± 0.06	1.18^{e} ± 0.06	4.85^{f} ± 1.06	0.46^{e} ± 0.033	1.214^{b} ± 0.270	65.42^{ab} ± 4.30
Syzygium cumini	11.02^{c} ± 0.94	3.92^{a} ± 0.47	1.05^{f} ± 0.04	5.28^{ef} ± 1.07	0.33^{fg} ± 0.026	0.044^{e} ± 0.031	68.09^{a} ± 3.62
Azadirachta indica	11.17^{c} ± 0.58	2.52^{c} ± 0.19	1.96^{b} ± 0.05	5.49^{ef} ± 0.85	0.82^{c} ± 0.019	0.156^{de} ± 0.102	45.08^{c} ± 5.05
Clerodendrom inerme	11.38^{c} ± 0.33	2.36^{c} ± 0.06	1.61^{c} ± 0.07	5.06^{ef} ± 0.65	0.17^{h} ± 0.016	1.462^{a} ± 0.392	63.28^{b} ± 2.61
Conocarpus erectus	10.21^{c} ± 1.55	3.60^{b} ± 0.33	1.39^{d} ± 0.04	16.17^{b} ± 0.85	0.24^{gh} ± 0.015	0.010^{e} ± 0.039	66.76^{ab} ± 1.31
Bougainvillea sp.	15.45^{b} ± 0.53	2.31^{c} ± 0.04	2.08^{b} ± 0.03	6.56^{de} ± 0.64	0.95^{b} ± 0.009	0.307^{d} ± 0.028	66.49^{ab} ± 0.68
Delonix regia	17.69^{a} ± 0.23	2.40^{c} ± 0.04	2.17^{a} ± 0.07	8.91^{c} ± 0.75	0.20^{h} ± 0.015	0.608^{c} ± 0.014	31.97^{d} ± 1.64
Dodonaea viscosa	15.37^{b} ± 0.20	2.34^{c} ± 0.00	1.61^{c} ± 0.06	24.94^{a} ± 1.52	1.14^{a} ± 0.160	0.054^{e} ± 0.016	68.36^{a} ± 1.17
Phoenix sp.	15.30^{b} ± 2.93	2.28^{c} ± 0.01	1.07^{f} ± 0.05	6.35^{d-f} ± 0.80	0.65^{d} ± 0.028	1.144^{b} ± 0.058	49.27^{c} ± 2.90
Means with different letters on the same column are significantly different ($p < 0.05$) based on LSD test							

Within the selected ornamental plants, the highest concentrations of ascorbic acid or vitamin C (8.50 mg/100 g F.W.) was noticed in *S. cumini* followed by *D. viscosa* (8.16 mg/100 g F.W.). The rate of vitamin C (4.93 mg/100 g F.W.) in *F. elasica* was low. Maximum amount of chlorophyll b (7.63 µg/ml), and flavonoid (3.92 M/g) was also extracted from *S. cumini*, while the amount of anthocyanin, and total protein was minimum in this species. The results showed that the levels of flavonoid in all ornamental species except for *S. cumini* and *C. erectus* ranged between 2.28–2.52 M/g. *D. regia* had highest amount (2.17%) of total protein and high amount (17.69 mg/100 g F.W.) of anthocyanin. This species was relatively poor in ascorbic acid. *Bougainvillea* sp. was a species with high content (2.08%) of total protein, and lowest content (3.54 µg/ml) of chlorophyll b. Also, *C. erectus* and *A. indica* had minimum anthocyanin and chlorophyll a. On the other hand, the concentration of flavonoid in *C. erectus* was high, but the concentration of carotenoid was low (Table 5).

Synthesis of osmolytes (carbohydrates and proline) increased considerably in response to excess of Co and Sn concentration in leaves. *D. viscosa* was found to contain most total soluble carbohydrates (24.94%) and proline (1.14 µM/g F.W.) concentration followed by *C. erectus* for total soluble carbohydrates and *Bougainvillea* for proline (Table 5). Total soluble carbohydrates and proline contents were affected significantly by HMs and differences between selected ornamental plants was significant (Table 4). Total soluble carbohydrates and proline contents significantly decreased in *C. inerme* and *F. elastica* with the highest activity of SOD and POD. *F. elastica*, *C. inerme*, *S. cumini*, and *A. indica* included the lowest total soluble carbohydrates content. Also, *C. inerme*, *D. regia*, and *C. erectus* synthesized least proline.

Results related to antioxidant enzymes activity (SOD and POD) in leaves of selected ornamental plants are presented in Tables 4 and 5. There was a significant difference in SOD and POD activities in leaves of these plants (Table 4). High concentrations of As, Pb and Sn in leaves of *C. inerme* and *F. elastica* caused a significant increase in SOD and POD activity. The activity of POD (1.462

and 1.214 U/g F.W./min) was highest in leaves of *C. inerme* and *F. elastica*, respectively (Table 5). SOD activity (63.28 and 65.42 U/g F.W.) was also high in leaves of these two ornamental species. POD activity was lowest in leaves of *C. erectus* (0.010 U/g F.W./min), *S. cumini* (0.044 U/g F.W./min) and *D. viscosa* (0.054 U/g F.W./min). A statistically significant difference in SOD activity was recorded in the leaves of selected ornamental plants (Table 4). The highest SOD activity (68.36, 68.09 and 66.76 U/g F.W.) was noticed in *D. viscosa*, *S. cumini* and *C. erectus*, respectively. The results presented in Table 5 indicated that these species containing the highest SOD activity had the lowest POD activity. The lowest SOD activity (31.97 U/g F.W.) was obtained in *D. regia*. The activity of this enzyme was low in *A. indica* and *Phoenix* sp., as well.

Discussion

The environmental pollution of HMs and their accumulation in food chains due to their adverse effects on living organisms is one of the main environmental problems of modern societies. The main sources of pollutions are water, soil and air that the main contaminants of these sources are urbanization and industrialization (anthropogenic activities). Metal toxicity causes the formation of free radicals (particularly ROS) which causes DNA damage, change in enzymes activity, alteration of homeostasis, and LPO (Asati et al. 2016). HMs have an adverse effect on most intra-cellular organelles, including mitochondria and chloroplasts. Many HMs are also toxic to humans in addition to plants, and four HMs; Hg, As, Cd and Pb have respectively the highest environmental risks due to their widespread use and extensive toxicity and distribution (Briffa et al. 2020). The city of Bushehr is exposed to some environmental pollutions for specific environmental and climatic reasons, including pollution from HMs. Phytoremediation approach was used in this study to reduce the contamination of HMs.

The high concentration of HMs in some species is closely related to the pollutants in water, soil, and air mainly because of human activities. Many studies showed that environmental pollution associated to HMs is highly correlated with traffic (Delaman and Farahmand 2017). The HMs effect is species- and dose-dependent. Some plants are able to accumulate HMs at high levels without serious injury. Only long-term exposure or exposure to high concentrations of HMs cause significant injury in some plants. Therefore, these types of plants must be detected in each urban area. In the present study, we selected the top 10 ornamental plants in Bushehr based on beauty, abundance, architecture, growth, being local and resistance to kinds of biotic and abiotic stresses to evaluate the amount of HMs accumulation in the leaf and physiological and antioxidant enzymes changes resulting from the presence of these HMs. The results showed the obvious difference between the selected species in relation to the accumulation of HMs and physiological and enzymatic parameters, including defense processes.

Phytoremediation (the use of plants to cleanse different environments), as the best bioremediation approach, is the most appropriate way to reduce HMs contamination in cities. Suitable plants for phytoremediation have high transmission and accumulation abilities of HMs (Briffa et al. 2020; Liu et al. 2021). The tolerance and resistance of plants against HMs or avoiding them is defined as the ability to survive in the soils that are toxic for other plants. Suitable plants for use in phytoremediation must have characteristics such as high tolerance ability to metals, extensive root system, absorption and accumulation of high metals in roots and shoots, high biomass production, rapid growth, resistance against pests and diseases, and appropriate in order to use as phytoaccumulation and phytoextraction strategies (Delaman and Farahmand 2017; Ghafari et al. 2020). Removing these metals from cytosol and entering them into vacuole and/or cell wall using chelating agents (ligands), especially organic acids and amino acids, the use of enzymatic and non-enzymatic antioxidants, and etc. are the most important protection mechanisms of plants against metal toxicity. Unlike the northern cities of Iran, Bushehr does not have much plant diversity. Among a few dozens of native plants in Bushehr, 10 dominant and abundant tree and shrub ornamental plant species were evaluated to investigate the amount of HMs within their tissues and ultimately the selection of superior plants in terms of absorption of these metals. Our findings showed that *C. inerme*, *F. elastica* and *Bougainvillea* had the highest concentration of HMs with more toxicity in their leaves. The high accumulation of HMs in trees and shrubs in urban green space indicates the high ability of these plants to harvest and absorb these contaminants from the surroundings (Delaman and Farahmand 2017). Different absorption and accumulation of HMs by different plant species and the use of superior species in urban green spaces for phytoaccumulation and phytoextraction strategies has been shown in many studies (Cameron et al. 2012; Sardabi et al. 2013; Delaman and Farahmand 2017; Edelstein and Ben-Hur 2018; Amiri et al. 2019; Conversa et al. 2019; Ghafari et al. 2020).

The presence of HMs on a higher scale is toxic to the plants. Also, the plant adopts strategies to reduce the effects of the toxicity resulting from the increase in these metals. In both of these cases, physiological and enzymatic changes occur in plant cells, some of which were observed in the present study. These changes are aimed at reducing the toxic effects of HMs or adaptation, tolerance and resistance to these metals. HMs induce morphological, physiological, biochemical and anatomical changes in plants (Upadhyaya et al. 2014). The most common response of plants to stress conditions, such as HMs, is growth reduction. Effect of HMs on plant growth and development is due to the interference of HMs with the biochemical processes, such as protein (enzyme) and chlorophyll biosynthesis (Parlak 2016). Studies indicates that tolerance to HMs is plant species- and even organ-dependent. Plants have some strategies to maintain and regulate cellular metal homeostasis by preventing uptake and accumulation of high concentrations of free HMs ions (Saidi et al. 2013; Ali et al. 2014). HMs affect the plants by lowering the photosynthetic pigments content (Quartacci et al. 2000; Upadhyaya et al. 2014; Wala et al. 2022) and stimulating the biosynthesis of some secondary metabolites (Shi et al. 2018). Pigment content and photosynthesis in various type of plants are generally reduced by metal stress. Current study showed reduced content of chlorophyll a, total chlorophyll, carotenoid, anthocyanin, and flavonoid in *C. inerme* and *Bougainvillea*. These pigments were in high concentrations in *F. elastica*. Our findings revealed different strategies of each species against high levels of HMs. Similar findings were reported by some other researchers. Reduction in chlorophyll a, b, total chlorophyll and carotenoid content was observed in shoot of *Vigna radiata* (Upadhyaya et al. 2014) and *Hydrilla verticillata* (Srivastava et al. 2013) at higher doses of As treatment. Decrease in chlorophyll and carotenoid biosynthesis could be due to the generation of ROSs like superoxide and hydroxyl radicals, and H_2O_2 that have the potential to damage proteins, nucleic acids and amino acids involved in the biosynthetic pathway of chlorophyll and carotenoid biosynthesis (Dat et al. 2000). Increased pigments content may be an adaptive response to cope with stress induced photosynthetic damage (Upadhyaya et al. 2014). Decline in chlorophyll levels could be due to a reduced efficiency of enzymes involved in chlorophyll biosynthesis, low Fe content, and replacement of central Mg^{2+} molecules in chlorophylls by the HMs (Dhir et al. 2009). Plants treated with Ni showed decreased chlorophyll contents in the leaves (Parlak 2016). The content of soluble sugars is generally changed during exposure of plants to various stresses particularly HMs such as Cd (Godinho et al. 2018). Changes in the content of some primary and secondary metabolites were observed in the presented study. Similar findings were reported in some plants (Godinho et al. 2018). Cd toxicity caused a significant decrease in plant growth characteristics, biomass (fresh and dry weights), and photosynthetic parameters (mainly with effect on pigments and activity of key enzymes) and intensively increased Cd concentration in different plant parts of *Gossypium hirsutum* L. (Farooq et al. 2016). HMs stress caused low soluble protein contents in some species due to more oxidative stress (Ali et al. 2014; Farooq et al. 2016).

Some HMs such as Cu, Zn, Fe, and Co are essential elements for plants, but increase in their levels may disturb physiological processes. The presence of Zn is essential to neutralize the toxic effects of Cd. Application of Co and Ni at proper concentrations had suitable effect on increasing fresh and dry weight and status of N, P, K, Co, Ni, Mn, Zn and Cu in *Hibiscus sabdariffa* L. (Eman et al. 2007). Co and Ni at the lowest level induced the quantity of anthocyanin and flavones. Co, at 7.5–12.5 mg/l, increased the content of N, P, K, Zn, Cu, dry weight, protein, total carbohydrate, total soluble solids, total phenolics, carotenoids, and vitamin C in leaves of *Moringa oleifera* Lam. In our work, *C. erectus* and *D. viscosa* with the highest content of Co had high relative water, vitamin C, and flavonoid. In some studies, Co at higher doses showed reduced stimulating effects (Gad et al. 2019). These researchers also showed the antagonistic relationships between Co and Fe. Ni at high concentration had unsuitable effect on photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoid) and dry matter yield in *Cicer arietinum* L. varieties (Batool 2018). Our results confirmed these findings, as the lowest rates of chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, anthocyanin, total soluble carbohydrate and proline was obtained in *C. erectus* and *C. inerme* containing the highest rate of Ni. Reduction in chlorophyll contents might be due to inhibition of enzymes for chlorophyll biosynthesis and decreased nitrogen contents. This reduction in photosynthesis adversely affected the growth and biomass (Batool 2018). Study on *Triticum aestivum* L. revealed that Ni affects plant height, and chlorophyll and proline contents (Parlak 2016). The results indicated that the increase in the activities of the antioxidant enzymes were not sufficient to protect cell membrane against Ni toxicity. HMs toxicity disrupts the conversion of organic acids to other metabolites necessary for optimal growth and development. Application of glycinebetaine (GB) decreased Cd concentration in different organs of *Gossypium hirsutum* L., decreased production of electrolyte leakage, H_2O_2 and MDA contents, and led to a significant increase in photosynthetic pigments, protein contents and antioxidant enzymes (Farooq et al. 2016). Decrease in plant growth and biomass might be due to HMs-induced toxicity on photosynthetic apparatus (Farooq et al. 2016). The compounds like proline and GB increase the uptake of plants nutrients, and maintain the photosynthetic capacity under stressful conditions (Holmstrom et al. 2000; Shahbaz et al. 2011). Increased content of proline was observed in our experiments in *D. viscosa*, *Bougainvillea*, and *A. indica*. Proline plays an important role in response to HMs stress. HMs toxicity induce proline accumulation in plants as a result of accumulation of ROS (Parlak, 2016). Proline also binds to metal ions.

HMs toxicity caused changes in the activities of antioxidant enzymes through the activation of oxidoreductases. Enzymatic and non-enzymatic antioxidant potential of some plants may be attributed to protects cells from HMs-induced oxidative damage (Upadhyaya et al. 2014). Toxic doses of some HMs stimulate biosynthesis of phenolic compounds and ascorbic acid in some plants, which is often associated with detectable increase in antioxidant potential of plant tissues (Muzolf-Panek et al. 2017). In current study, high levels of As, Pb and Sn in leaves of *C. inerme* and *F. elastica* caused a significant increase in SOD and POD activities. The activity of POD was highest in leaves of *C. inerme* and *F. elastica*. SOD activity was also high in leaves of these two species. Our findings showed that these species with the highest SOD activity contained the lowest POD activity. Arsenic reduced root growth in mung bean due to oxidative stress resulting from enhanced LPO (Singh et al. 2007). The activity of SOD, POD, CAT and APX was increased in *Gossypium hirsutum* L. grown under lower Cd stress while decreased under higher Cd stress. Cd toxicity increased the concentration of ROS as indicated by the increased production of MDA, H_2O_2 and electrolyte leakage in both leaves and roots (Farooq et al. 2016). Decrease in plant growth and biomass during excess of HMs might be due to oxidative damage and reduction in antioxidant enzymes activities and reduction in mineral nutrients uptake by plants (Hediji et al. 2015). In our study, least dry matter was observed in *C. inerme*. Enhancement of antioxidant enzymes activities at lower Cd stress might be in order to the activation of plant defense mechanism (Ali et al. 2014). Antioxidant enzymes like SOD and POD play an important role in reducing the MDA contents under metal stress (Ali et al. 2014). On the other hand, reduction in activity of antioxidant enzymes at higher Cd stress might be due to the breakdown of ROS homeostatic balance, followed by increase in H_2O_2, MDA contents and electrolyte leakage (Saidi et al. 2013; Farooq et al. 2016). MDA content is an indicator of oxidative stress. HMs induce plant LPO due to the production of free radicals such as OH^+, or by lipoxygenase (Parlak 2016). The present study revealed that there was a reversible relationship between the concentration of osmolytes (carbohydrates and proline) and the activity of the antioxidant enzymes (SOD and POD) in selected ornamental plants under the stress of HMs toxicity, so that plants with high osmolytes concentrations had low enzymatic activity and vice versa. Perhaps one of the reasons for this approach is to save energy during HMs-stress conditions. *C. inerme* and *F. elastica* which had the highest activity of the antioxidant enzymes, contained the lowest concentration of osmolytes in their leaves tissues.

The type of plant, especially plant phenology, has an effective role in the absorption and accumulation of environmental pollutions, including HMs. This was also shown in the present study, so that selective shrubs were more appropriate than the chosen trees to absorb more HMs from the environment. Shrubs are more suitable than trees probably because of having more superficial and more extensive root system.

Overall, high levels of HMs are toxic to plants and human. HMs toxicity affect different physiological, morphological, and anatomical processes in plants. HMs disturb plant water relations. At cellular level, these metals change structure, configuration and functions of organelles such as vacuole, endoplasmic reticulum, nucleus, Golgi apparatus, chloroplast, and mitochondria. HMs decrease the content of photosynthetic pigments particularly chlorophyll. ROS is produced in response to HMs stress. Excess of ROS causes lipid peroxidation, damage to nucleic acids, and modification of enzymes and other proteins as well carbohydrates. In this condition, the content of MDA, and activity of antioxidant enzymes such as CAT, SOD, POD, and APX increase. One of the most important ways to reduce the content of HMs from human living environment especially in cities is the use of ornamental plants with high capacity for uptake of HMs. We selected 10 dominant ornamental plants grown in Bushehr to compare their ability for absorption of 10 HMs and their influence on some physiological response and antioxidant enzymes activity in order to the expansion of green spaces. Results showed that *C. inerme* contained the highest content of As and Sn. POD activity was highest and SOD activity was high in this species. *Bougainvillea* had maximum amount of Zn, Cu, and Cr. SOD activity was high in leaves of this species. The highest amount of chlorophylls, carotenoid, and anthocyanin, as well high activities of SOD and POD were obtained in *F. elastica*. Biosynthesis of osmolytes increased in response to excess of Co and Sn contents. *D. viscosa* included the highest content of total soluble carbohydrates and proline. Total soluble carbohydrates and proline contents significantly decreased in *C. inerme* and *F. elastica* contained with the highest activity of SOD and POD. Overall, we recommend three shrubs, *C. inerme*, *F. elastica* and *Bougainvillea* for green space of Bushehr and other cities all over the world with similar climatic and geographic conditions.

Declarations

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Authors’ contributions All authors contributed to the idea, design and analysis for the study. RA doing project and capturing photos, BK methodology, investigation, and writing-original draft, SS review and editing and visualization, MSA formal analysis and review and editing.

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Figures

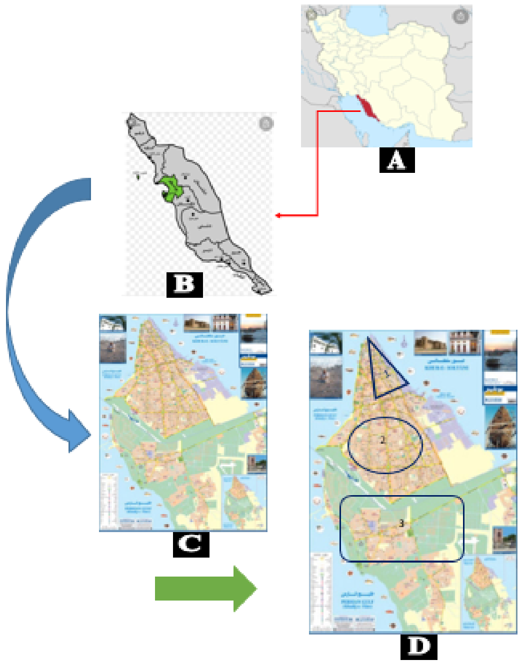

Figure 1

Site of study. The map of (A) Iran, (B) Bushehr Province, (C) the city of Bushehr, and (D) three studied regions in the city of Bushehr

Figure 2

Images of 10 selected ornamental plants with the highest frequency. A: Sacred fig (*Ficus religiosa*), B: rubber fig (*Ficus elastica*), C: jaman or jambolan (*Syzygium cumini*), D: neem or Indian lilac (*Azadirachta indica*), E: sorcerers bush or wild jasmine (*Clerodendrom inerme*), F: button mangrove or buttonwood (*Conocarpus erectus*), G: bougainvillea (*Bougainvillea* sp.), H: flamboyant or royal poinciana (*Delonix regia*), I: sticky hop bush (*Dodonaea viscosa*), and J: palms (*Phoenix* sp.)