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An Examination of Antioxidant, Physiological and Chemical Responses of Olive, Rose and Pine, Under the Stress of Air Pollution in Isfahan

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Since different plants have different reactions to pollutants, in this study the physiological, chemical and Antioxidant changes of three plants, Olive (broadleaf), Rose (shrubs) and Pine (conifer), affected by urban (traffic) and industrial (petrochemical) pollution, compared to the control group (in Semirom), were studied. Leaf samples from the three plants were collected randomly during the two different periods (June and July). The results showed significant differences in chlorophyll, carotenoids, anthocyanins, the number of stomata in the three plants Rose, Olive and Pine in the three regions (P <0.01). Protein content in the Soffeh and petrochemical regions decreased compared to Semirom, and catalase activity increased significantly. Ascorbate peroxidase enzyme activity differences among the plants in the regions, at 0.01, was not significant. The results showed significant differences in the concentration of zinc and copper inRose, Olive and Pine was found in thethree locations (P <0.01). The soils of all three regions were diagnosed withlead elements and the soil of Soffeh and Petrochemical plant were found to bepolluted by zinc. The results indicate physiological, chemical and Antioxidant adjustments in the three plants and, consequently, the possibility of using all three plants of Roses, Olive and Pine as aliveindicators of air pollution.

Key words: Antioxidant, physiological, chemical, olive, rose, pine, pollution

Definition of the Problem

Air pollutants include any type of gas, liquid, solid, or mixes of them whichs pread in free air and cause air pollution, or adds to the pollution or creates unpleasant odors[1]. Contamination of agricultural products and plants with heavy metals -which are defined as metals with a density greater than 5 grams per cubic centimeter, which, because of their toxicity, high degree of persistence, potential bioaccumulation and non-degradability, are of the most important compounds in different ecosystems[2] - on the one hand causes a decrease in the quality of agricultural products (as a major source of food) and on the other, is a serious threat to human health. The accumulation of these pollutants and the increase in their concentration to the point of danger, can threaten human health by entering the food chain.

Olive

Olive,an evergreen tree with the scientific name Oleaeuropaea, is of the branch of angiosperms (flowering plants), is of polypetalous and of the order of Lamialesand of the olive family. About 20 species of Olives have been identified in various regions, including tropical and semi-tropical regions[3].

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Pine

The subspecies of pine belongsto the order ofPinusand Pinal family which includes 9 genera and 300 species. This species' important characteristics is havingneedle-like leaves which are arranged spirally on the shoot or bundled in clusters, are evergreen but in some genera deciduous, and they are arranged in a cyclical pattern of dark green covered with pale dust, with a length of 8 to 15 centimeters[4].

Rose

Roses are shrubs from the rose family, of the genus Rosa, with more than 2000 species and approximately 100 genera. Rose flowers are small in sizes, and the height of the flowers can reach up to 7 meters[4].

History of Science

The air pollutants' effects on plants have been known since long ago. In 1999 it was reported that substitution of zinc, copper, mercury, nickel and lead rather than magnesium in chlorophyll causes a fracture in photosynthesis[5]. Studies on Samar (Prosopisjuliflora) tree's leaves which have been exposed to industrial pollution in the air (around an oil field in southwestern Iran), showed that in the exposed sample leaves that were exposed to oil contamination,peroxidase and catalases activity increase significantlyand the protein content in these regions decreased in a significant amount. Increased levels of ascorbic acid in the contaminated areas were also observed[6]. Results of study on wheat and mustard grown in the vicinity of industrial and urban areas showed a decrease in total chlorophyll content, carotenoids, ascorbic acid, plant height, fresh stem weight, fresh root weight and yield in affected areas compared to safe or control areas[7]. Research on acacia plants like Oleander and Acacia in Tehran represents a higher level of peroxide and catalase plant enzymes in leaf samples collected from the infected area (South Liberty Park) in comparisontothe healthy area (SorkhehHesar park). The higher levels of the peroxidase enzyme activity in acacia RobiniaPseudoacaciin comparison to the control group ($p < 0/05$) were statistically significant. This study showed that both Acacia and Oleander can be considered as effective living indicatorsthat reflect air quality in areas[8]. Research results on physiological and morphological responses ofolive tree to ozone showed that in leaves that were soaked in ozone the number of stomata decreased. Also, in leaves soaked in o3, a significant reduction in photosynthetic activity(compared to control sample) was found[9].During the study that was carried out in three tree species (Silver Cypress, Pine and Oak) and three shrub species (Oleander, Sweet Barberry), it was concluded that in most cases, in terms of revealing the excess levels of metal contamination, trees arebetter than shrubs and evergreen plants are better than deciduous plants. The biggest amount of iron and nickel were found in the cypress leaves and pine bark. They showedsignificant differences in iron with all plants and in nickel with most plants[10].

TheImportance and Necessity

Since determining the effects of air pollutants, especially heavy metals, including lead, cadmium, copper and zinc on these plants -in order to estimate their physiological, chemical and antioxidants responses – from different environmental aspects and determining the resistance of each against pollution seems essential, in this research, pine (coniferous) is compared with hardwood trees (Olive) and shrubs (Rose) were analyzed under the stress of infection.

MATERIALSAND METHODS

Regions under Study

Using the information of environmental organizations of Isfahan, Semirom, SoffehForest Park andPetrochemical plant site were chosen asunpolluted, heavy traffic and industrial areas respectively. Leaf samples from plants in the three locations were collected simultaneously (July and September) in a randomized manner with three repetitions for each plant. From 0-30 cm depth, soil samples were taken from the areas in order to measure the concentration of chemical elements.

Measuring Psychological Properties

Chlorophyll a and b and total were calculated usingArnon relations[11] and the amount of anthocyaninswas calculated using Wagner's method[12]. Carotenoid concentrations were calculated as well[13]. New stomata in the leaves were found and counted, using a model BX-40 optical microscope manufactured by OLYMPUS co.which was equipped with a Canon video camera, with 10 and 40 zoom degrees[14]. Then, clearer images were chosenand transferred into the Adobe Photoshop

CS2 software and then into Micromager 3/3. Width and length of the stomata were measuredwith Micromager and was presented using EXCEL 2014.

.MeasuringConcentrations of Lead, Copper, Zinc and Cadmium in New Plant Tissue

To measure chemical elements lead, copper, cadmium and zinc,collected leaf samples were dried and pulverized.Then the amount of the elements in the resulting extract was measured by AAS atomic flame absorption (according to the method given by Agricultural Research Center)using the digestion method aided by sulfuric acid-salicylic acid-hydrogen peroxide, using Perkin-Elmer's atomic absorption device model 6030 manufactured in the United States[15]. To measure the absorption amounts of the heavy metals lead, zinc, cadmium, nickel and copper in the soil, absorbing of samples by a 0.005molar DTPA solution containing 0.01 molar calcium chloride with pH of7.3 was extracted and was measured with atomic absorption which was made possible by the Perkin Elmer Model 6030[16].

MeasuringAntioxidant Properties (Catalase Enzyme Activity and Ascorbate Peroxidase) and Protein Content

To measure the activity of the enzyme ascorbate peroxidase, the mixture of the chemical reaction contained ascorbate 5.0 mM in phosphate buffer, 1 mM H_2O_2, EDTA 0.1 mM in phosphate buffer and enzyme extract. To measure the progress in the enzymatic reaction, the UV visible device Spector Flex 6600 model was used[17].

Evaluation of Catalase Enzyme Activity

Evaluation of the activity of catalase enzyme (CAT) was done by studying the amount of hydrogen peroxide in a wavelength of 240 nanometers for duration of one minute. Mixture contained a reaction of 100 mM potassium phosphate buffer, pH = 7 and hydrogen peroxide 15 mM. The reaction was started by the addition of 100 mlM enzyme extract and 900 microliter of the reaction solution. The changes in absorbance at 240 nm were recorded for 90 seconds. The enzyme activity was calculated using the extinction coefficient of 0.036$mM^{-1}cm^{-1}$and was defined on the basis of one unit (micromole of hydrogen peroxide consumed per minute) per 1 mg protein[18].

Method of measuring protein

Macro BradfordProtein Assay was used for the measurement. To determine the amount of protein in leaf extracts, a series of test tubes was produced and 250 microliter of phosphate buffer, 250 microliter 1 molarNaOH solution and 5 milliliter of Bradford reagent was added to each tube. Then 500 microliter of the extract was added to each tube so that the final volume of each tube was 6 milliliter. The content within each tube was mix well by vortex and after 2 minutes absorption at a wavelength of 595 wasread.

Statistical analysis

Statistical analysis of the variance datacalculations was done using SPSS andSAS software, while comparison of the mean was done by using Duncan's multiple-range test and at 1% level, diagrams were drawn with EXCEL software.

Results

Comparison of the square mean of the analysis of the measured properties showed that the plants from the three regions there is a significant difference in psychological (the rate of chlorophylla, chlorophyllb, total chlorophyll, carotenoid and anthocyanin, the number and dimensions of stomata), the properties of antioxidants (catalase activity and protein content) and chemical properties (amount of calcium, zinc, copper and magnesium in the plant and zinc, copper, lead, cadmium, manganese and iron elements in the soil) of the plants at level 0.01, (Tables 1 to Table 14). The lead and cadmium content in the studied leaf samples were lower than the detection limit of the device.

RESULTS AND DISCUSSION

Evaluating the Results of Measurements (Chlorophyll A, B, and Carotenoid)

Based on the results the amount of chlorophyll a, b, and Carotenoid in the olive and pine samples from Soffeh as well as Petrochemical plantdecreased compared with the control group (Semirom). These findings match the result of the study on Prosopis tree in industrial areas adjacent to the thermal power plant[19], and research on olive plant in urban traffic areas[20]. According to the report, the causes of oxidative stress decrease chlorophyll content by disrupting the return to balance in protein complex optical system 2[21]. By preventing the absorption of essential elements such as iron and magnesium, heavy metals stop chlorophyll synthesis and cause degradation of the photosynthetic apparatus due to shortage in protein ligands. In stress condition of air pollution enzymes involved in the synthesis of chlorophyll stop working, andas a result of chlorophyll degradation the frequency of heavy elements occurs[22]. Also, it is reported that a high concentration of metals that can cause destruction and disturbance of carotenoid structures and so reduction in number of those plant[23]. In the case of Rose shrubs in Soffeh, an increase in chlorophyll a, b, total and carotenoidwas observed in comparison to roses of the control group (Semirom) which match the study[24]. It seems that the increase in pigment content of leaves during high

Table 1. Mean Squaresof the physiological characteristics of olive, rose and Pine

S.O. V	Square Mean			
	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Carotenoid
Treatment	0.576**	0.718**	0.287**	2.831**
Error	0.002	0.0004	0.0001	0.00009

** Indicates a significant difference at 1% level

Table 2. Mean Squaresof the physiological characteristics of olive, rose and Pine

S.O. V	Square Mean			
	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Carotenoid
Place	0.972**	1.260**	0.456**	5.792**
Genotype in Place	0.406**	0.486**	0.214**	1.562**
Error	0.002	0.0004	0.0001	0.00009

** Indicates a significant difference at 1% level

Table 3. Comparison of the physiological characteristics Mean in three plants

Treatment	Chlorophyll A mgg- 1 FW	Chlorophyll B mgg- 1 FW	Total Chlorophyll mgg- 1 FW	Carotenoid µ g g -1 FW
kse	1.702[a]	1.965[a]	1.115[a]	3.886[a]
zse	1. 286[b]	1.019[b]	0.449[c]	2.953[c]
rse	1.162[c]	0.740[d]	0.253[e]	2.617[d]
kp	0.303[g]	0.248[h]	0.113[gh]	0.695[k]
zp	0.699[e]	0.377[g]	0.095[h]	1.470[i]
rp	0.822[d]	0.449[f]	0.116[g]	1.652[g]
ks	0.738[e]	0.436[f]	0.057[i]	1.739[f]
zs	0.622[e]	0.346[g]	0.093[h]	1.336[j]
rs	1.639[a]	0.988[b]	0.310[d]	3.327[b]

Similar letters in each column indicate that there are no significant differences at1%level .

Table 4. Means Squars of AnthocyaninConcentration

S.O. V	D. F	Mean Square Anthocyanin
Treatments	8	$5.37 * 10^{-10}$ **
Error	18	$9.94 * 10^{-13}$ **

** Indicates a significant difference at 1% level

Table 5. Means Squars of AnthocyaninConcentration

S.O. V	D. F	Anthocyanin
Place	2	$5.98*10^{-10}$ **
Place in Genotype	6	$5.16*10^{-10}$**
Error	18	$1.79*10^{-11}$

** Indicates a significant difference at 1% level concentrations of heavy metals can be attributed to the accumulation of pigments in undeveloped leaves due to metal toxicity[25]. While a low concentration of metals stimulates synthesis of chlorophyll and photosynthetic activity and increase growth as a result[26]. Since the results from a study of heavy metals copper, iron, zinc, etc. measured in this study showed higher concentrations of these elements in Soffeh, it may be concluded that the stress of high concentrations of metals, act as the limiting factor for the synthesis of chlorophyll and carotenoid in this plant (SoffehRose)[25].

Table 6. Comparison of anthocyanin concentration mean

Treatments	Anthocyanin mMg^{-1}
rse	$4.583*10^{-5}$ a
zse	$7.55*10^{-6}$ d
kse	$1.76*10^{-5}$ b
rp	$9.87*10^{-6}$ c
zp	$1.03*10^{-5}$ c
kp	$6.43*10^{-6}$ d
rs	$9.55*10^{-6}$ c
zs	$2.19*10^{-6}$ e
ks	$2.18*10^{-6}$ e

Similar letters in each column indicate no significant differences at 0.01level.

Table 7. Mean squares of the stomatanumber

S.O.V	D. F	Mean Square Number of Stomata
Treatments	8	56.92**
Error	81	1.391**

** Indicates a significant difference at the level of 1 percent.

valuation of the Results of Anthocyanin

Based on the results, the amount of anthocyanin in all plants in Soffeh and Petrochemical plant (except olive in the latter area) decreased compared to samples fromSemirom. A study of Albizialebbeck and Indian oleandershowed anincrease in anthocyanin in a rate of 8-35% in traffic pollution, as well[27], which is consistent with current results. But the compared to Semiromolive,olives in Petrochemical plant had anincrease in anthocyanin content was observed which matched the results the study on Purslane, which indicated a rise in anthocyanin and carotenoids, under copper stress[8].

Evaluating the Results of Stomata

The Number of Stomata

Evaluation of the results of the number of stomata represents a reduction in the number of stomatain rose and olive inSoffeh and petrochemical plant areas compared to the control

Table 8. Mean squares ofcatalase and peroxidase enzyme activity and protein content

S.O. V	D. F	Mean Square		
		Protein	Catalase	Peroxidase
Treatment	8	0.018**	0.057**	0.0000014^ns
Error	18	0.001	0.0025	0.000001

** Indicates a significant difference at 1% level, and ns indicates “no significance”.

Table 9. Mean squares of catalase and peroxidase enzyme activity and protein content

S.O. V	Protein	Catalase	Peroxidase
Place	0.011**	0.075**	$1.338*10^{-6}$ ns
Place in Genotype	0.018**	0.065**	$1.512*10^{-6}$ ns
Error	0.001	0.005	$1*10^{-6}$

** Indicates a significant difference at 1% level, and ns indicates “no significance”.

Table 10. Comparison of the means of catalase, peroxidase Enzyme Activity and protein content

Treatments	Peroxidase	Catalase	Protein
rs	0.0009[a]	0.365[b]	0.803[a]
ks	0.0004[a]	0.026[f]	0.711
zs	0.001[a]	0.281[c]	0.594[de]
rse	0.001[a]	0.027[f]	0.744[a]
kse	0.0008[a]	0.016[g]	0.165[cde]
zse	0.001[a]	0.143[e]	0.624[cde]
rp	0.002[a]	0.277[b]	0.646[cd]
kp	0.0005[a]	0.026[f]	0.672[bc]
zp	0.002[a]	0.361[a]	0.577[e]

Similar letters in each column indicate no significant differences at 1%level.

group (Semirom) which match results of studies on the effect of air pollution on acacia[28]. It has been reported that air pollution reduces gas exchange in the leaf surface and closes the stomata and reduces the rate of photosynthesis. But a small increase in the number of stomata in petrochemical plant's pines in comparison toSoffehpine which match the results of the study on soybean under cadmium stress[29].

The Length of the Stomata

Based on the results of stomata length measurement, the stomata in the three petrochemical plants (rose and Pineand olive)increased in length compared to the same plants from Semirom. But the increase in pine and

Table 11. Mean Square of heavy metals concentrations in plant

S.O. V	D. F	Square Mean of Elements in the Plant					
		Ca	Mg	Cd	Pb	Zn	Cu
Treatment	8	0.624**	0.075**	0.00	0.00	4048.04**	24.064**
Error	18	0.019	0.037	0.00	0.00	3.52	3.037

** indicates a significant difference at 1%level.

Table 12. Mean Square of heavy metals concentrations in plant

S.O. V	Calcium	Magnesium	Zinc	Copper
Place	0.077*	0.08[ns]	6326.9**	6.703[ns]
Place in Genotype	0.807**	0.07[ns]	3288.4**	29.85**
Error	0.01	0.03	3.51	3.03

* Indicates a significant difference at 5% level, ** indicates a significant difference at 1% and ns indicates "no significance".

Table 13. Comparison of the Means of heavy Metal Concentrations in plant

Treatments	Ca	Mg	Cd	Pb	Zn	Cu
zp	1.710[a]	0.166[b]	0.00	0.00	37.667[e]	15.667[a]
kp	0.433[d]	0.503[a]	0.00	0.00	45.000[c]	11.000[c]
rp	0.523[cd]	0.436[ab]	0.00	0.00	29.333[f]	13.667[abc]
zse	0.743[c]	0.130[b]	0.00	0.00	41.333[d]	14.667[ab]
kse	0.336[d]	0.110[b]	0.00	0.00	53.667[b]	14.333[ab]
rse	1.203[b]	0.303[ab]	0.00	0.00	29.667[f]	6.3333[d]
zs	1.130[b]	0.123[b]	0.00	0.00	52.667[b]	12.000[bc]
ks	0.556[cd]	0.123[b]	0.00	0.00	53.667[b]	14.667[ab]
rs	1.140[b]	0.413[ab]	0.00	0.00	149.33[a]	12.333[bc]

Similar letters in each column indicate no significant differences at 1%level.

Table 14. Mean squares of heavy metals concentration in soil

S.O. V	D. F	Square Mean (Soil)					
		Cd	Pb	Zn	Cu	Mn	Fe
Treatment	2	0.00581**	5.16**	8.639**	2.777**	30.124**	28.16**
Error	6	0.00019	0.000199	0.00030	0.00014	0.0079	0.0001

** Indicates a significant difference at 1%level

Table 15. Permissible limit of soil elements

	Maximum (mg/kg)	Minimum (mg/kg)	Critical (mg/kg)
Absorbable Copper	8.64	0.36	0.8-2.5
Absorbable Zinc	67.20	0.98	1-3
Absorbable Cadmium	0.19	Negligible	

olive was not significant compared to pine and olive in control group. Similarly, a slight increase in stomata lengthin the Soffeh rose was observed which was not significant. While,compared with control sites, a significant decrease in stomata length of the Soffeholive was observed, Soffeh pine showed a slight decrease in the stomatalength compared to rose and pine in control group which was not significant. Possibly,perceived stress decreased stomatal openness in the parts related to air and the leaves and the subsequent increase in stomatal resistance[30]. Closure and reduction in the size of stomata in olives undercement stress was reported[31] which is in accordance with a section ofthe results from of this research.

The absence of significant changes, along the length of the stomatain petrochemical and Soffeh pines compared toSemiromis probably due to the high resistance of the plant due to its lesser stomatal conductance[32].

Stomata's Width

Studying the results of stomata width measurement showed a decrease in this parameter in Soffeh's olive and pine as well as petrochemical pine compared to the plants from control area, which matched the research on ViciaVillosa under copper and cadmium stress[32]. Possibly due to urban pollution (traffic, vehicles) and also due to higher levels of heavy metals measured in Soffeh,the reduction in the size of the stomata in the leaf samples has been a strategy for coping with pollution stress. In contrast, the petrochemical area has been devoid of urban traffic andindustrial pollution has been reported with lower levels in this site.

Evaluation of the Results of Measuring Antioxidant Properties

Protein Content

The results of the measurements show that the protein content of rose and olive in the petrochemical area and olives in Soffeh, showeda decrease in their protein content compared to the control group. The results of the study on Prosopisjuliflora[6] and the study on the effects of chromium contamination of sunflowers[25] match these findings. According to reports, the reduction in protein content during pollution stress can be linked to reduced synthesis of some proteins or increase in proteolytic enzyme activity[33]. But petrochemical and Soffeh pines increased their protein contents compared to the control pine. Ewais showed that the concentration of cadmium, nickel and lead, increased the amount of proteins and enzymes in some weed species[34]. The reason may be the synthesis of amino acids and protein in the leaves, as one of the important mechanisms of plants in reducing the toxicity of pollutants[35].

Catalase Activity

Based on the evaluation of catalase activity, although the increase of this enzyme in Soffeh and petrochemical pine was not significant compared to Semirom, an increase in catalase activity in all three plants from the two sites was observed in comparison to plants from Semirom.

The result of the final report of Mousavi *et al.*,[6], on Samar tree plant Prosopisjuliflora exposed to oil pollution, matches these results. According to research, under air pollution and oxidative stress, plants use enzymatic (catalase, peroxidase, etc.) and non-enzymatic (antioxidants such as anthocyanins And carotenoid) immunity systems to protect themselves[36].

Evaluating the Results of the Measurement of Chemical Properties

Zinc in Plants

The concentration of zinc is less than the permissible limit in all plant samples except Soffeh rose. This is in line with the results from research on acacia [10].

Copper in Plants

For copper in plant tissues, the minimum permissible limit is 5-30 micrograms per gram and the critical limitis passing 20-30 microgram per gram, has been determined[37]. In terms of copper concentration, all the studied plant samples have been lower than the criticallimit.

Cadmium in Soil

Since the permissible limit of cadmium in the soil is determined to be (0.01 - 3) $\mu g g^{-1}$ [37], it can be noted that in this study, there are no cadmium contamination in the soil of the studied areas. This matches the results of the study in the industrial area of Mobarakeh Steel Factory [10]. High levels of cadmium in Semirom's soilmay be attributed to the existence of parent materials.

Lead in Soil

Urban traffic has been claimed as the reason for the existence of additional lead in soil. The average concentration of this metal in all three soil samples in this research (Soffeh, petrochemical plant and Semirom), has been higher than the permissible limit. This report matches with the results of a study on acacia[10]. The average level of absorbable lead inSoffeh's soil (5.15)was determined to be higher than the concentrations of this element in the Petrochemical (3.66) and Semirom (2.35). Due to the heavy traffic of the Soffeh, the result is acceptable.

Copper Soil

Soffeh's soil has been observed to have the highest concentration of copper in the samples (2.31), while this amount has been 0.91 in thepetrochemical plant and 0.46 in Semirom. The higher amount of copper in Soffeh soil is most likely due to traffic. Copper pollution has not been observed in the soil of any of the studied areas.

Zinc in Soil

The average amount of absorbable zinc in Soffeh, petrochemical and Semirom areas was determined to be 3.63, 3.5 and 0.65 milligram per kilogram, respectively. The highest amount of absorbable zinc belongs to Soffehand the lowest belongs toSemirom's soil. The permissible limit of amount of absorbable zinc in soil is 0.98 - 67.20 and the critical has been determined to be 1-3 milligram per kilogram[38]. Thus,Soffehand petrochemical soil,arepolluted with zinc. This is in line with the results of the study by Haj Rasouliha et al. in 2006. According to reports, the origin of the excess zinc in soil can be a result of wear of tires and use of fossil fuels[39] (Table 15).

CONCLUSION

Generally, the results of this study showed different responses of the three plants, roses, olive and Pineunder pollution stress. Thus, it is possible to use these plants to reduce the effects of air pollution. Despite the soil pollutionofthe petrochemical plant andSoffehwith lead, lead contamination was not detected in any plant samples. Therefore all three of these plants can be used to removelead pollution from the environment. However, due to different reactions ofpetrochemical olive compared to Semirom's duringthe increase in anthocyanin, it may be noted that underpollution stress,olive combines stomatal or un-stomatal limiting systems tolimit photosynthesis. The cultivation of this plant in polluted conditions is highly recommended.

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