

Article

Evaluation of Bioindicator Interactions in *Cynodon dactylon* under Lead Stress

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Abstract: An experimental study was conducted on Bermuda grass (*Cynodon dactylon*) at four different sites in the Al-Bawiya area of Baghdad. The results showed a correlation between soil and root lead concentration ($r = 0.91$). A decrease in the transport factor (TF) (0.18–0.36) indicates the success of the plant exclusion strategy at moderately polluted sites (S1, S2). In contrast, the highly polluted site (S3) showed a significant decrease in the biocapacity factor (BCF) to 0.42, an increase in the transport factor (TF) to 0.36, and a decrease in the tolerance index to 58%. This is biologically attributed to damage to the root plasma membrane and the loss of selective barriers that allow lead accumulation in the leaves and energy diversion for growth in the face of oxidative stress. Lead accumulation exceeds the limits permitted by Iraqi and international law. Bermuda grass (*Cynodon dactylon*) proves to be an effective plant control agent in moderately polluted soils. However, it becomes ineffective in terms of protection and acts as a carrier of lead toxicity above 300 mg/kg.

Keywords: Lead Pollution, Phytostabilization Translocation & Bio-concentration Factors, Oxidative Stress & Physiological Breakdown

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1. Introduction

The contamination of heavy metals is among the most threatening ecological issues in urban environments, especially in cities that are subjected to inadequate solid waste disposal and sewage management systems. Pb is among the most toxic heavy metals in such contaminated soils, since its entry into the soil leads to changes in the physical and chemical composition of the environment and can harm living organisms through food chains [1]. Plants respond to heavy metal stress physiologically and biochemically in different ways, whether by excluding or accumulating the elements. *Cynodon dactylon* (Bermuda grass) is a widespread plant in Iraq, which has a deep tap root. Therefore, this plant can be considered as an ecological option for such polluted soils. Nonetheless, there is still an absence of information on the threshold of Pb tolerance in local contaminated soils and the ability of such a plant to restrict the translocation of the heavy metal without undergoing any cellular damage [2]. From past literature, it is clear that the plants follow elaborate protective measures to overcome lead stress based mainly on the "exclusion principle" [3]. In this context, Baker & Walker found that phytostabilizers depend on the presence of the selective barrier, i.e., the casparian strip to prevent heavy ions from accessing the vascular cylinder [4]. Biochemically, studies have confirmed that plants initiate lead chelation through phytochelatins and metallothioneins to sequester the same inside vacuoles. Analytically, USEPA method (USEPA 3050B) has established that the use of concentrated nitric acid in wet digestion technique helps extract total metals and 0.01M

EDTA in root washing serves as the best way to differentiate surface adsorption from bio-adsorption of lead in the soil [4]. However, there has been limited scientific investigation on the relationship between transmission coefficient (TF) and tolerance index (TI) quantitatively with respect to the ion mimicry phenomenon of lead at elevated levels [5]. This study intends to investigate the physiological and biochemical reactions of *Cynodon dactylon* under soil polluted with waste incineration products in the Al-Buwaya region of Baghdad, and the establishment of the critical concentration at which physiological dysfunction occurs through the calculation of TF, BCF, and TI indices. The levels detected are later compared with Iraqi and international standards to confirm their suitability for phytoremediation purposes.

2. Materials and Method

1. Experimental Setup and Sampling Method : A comparative field study has been performed to investigate the biological reaction of Bermuda grass *Cynodon dactylon* to lead (Pb) contamination. Four locations have been selected in the Al-Bawiya locality of Baghdad, Iraq depending on cumulative pollution trends because of waste burning and sewage discharge [6]. It has consisted of three polluted spots around pollution sources (S1, S2, S3), and one clean spot (C), which is situated in an ecologically secure zone without any anthropogenic pollution following standard procedures [7]. Soil samples have been collected from the root layer with a depth of 0-20 cm by means of a stainless-steel auger to avoid cross-contamination [8]. These soil samples have been air-dried, pulverized and sieved through a 2 mm sieve. Then whole *Cynodon dactylon* plants have been excavated from each location, and their roots have been thoroughly washed with 0.01M EDTA to strip adsorbed lead, followed by two washes with distilled water [9].

2. Chemical Analysis : The lead (Pb) content in the soil and plant samples was estimated according to the method of wet acid digestion used in the United States Environmental Protection Agency (USEPA) Method 3050B [10], 0.5 g of the sample was digested with 10 ml of conc. HNO₃ (Nitric Acid) at a temperature of 120 °C. The calibration curve is shown below in figure 1, and lead was estimated by using Atomic Absorption Spectrometry (AAS) (PerkinElmer AAnalyst 800) using air and acetylene flame. Certified reference material analysis ensures recovery values greater than 95% [11].

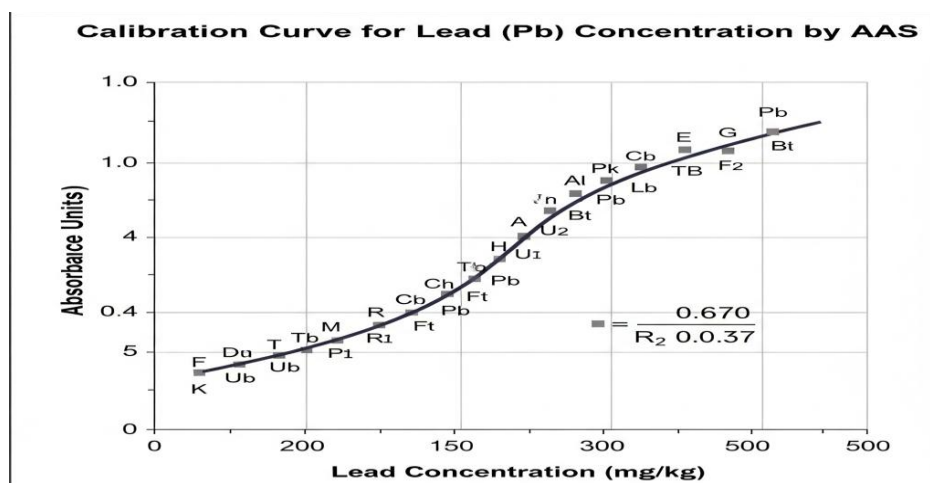


Figure 1. Calibration Curve for estimation of Lead (Pb) Concentration in Soil and Plant Samples Using Atomic Absorption Spectrometry (AAS).

3. Morphological and Physiological Investigation

The biological response measured includes the following:

- a. Biomass: Determination of dry weight. Estimated by drying samples for 48 hours at 70°C [12].

- b. Translocation factor (TF): A measure to determine the movement of lead from roots to the stem is given below:
- c. Tolerance index (TI): Percentage of dry biomass grown in contaminated and uncontaminated sites estimated as follows according [13]:

$$TF = \frac{[Pb]_{shoot}}{[Pb]_{root}} \quad TF = \frac{[Pb]_{root}}{[Pb]_{shoot}}$$

$$TI(\%) = \frac{DW_{sample}}{DW_{control}} \times 100 \quad TI(\%) = \frac{DW_{control}}{DW_{sample}} \times 100$$

4. Statistical Analysis: The statistical analysis used in this experiment was through one way ANOVA test to find the difference between different sites for their means, and then DMRT to find any statistically significant difference. Pearson correlation test was used to analyze the relationship between soil lead content, root lead content, stem lead content, and growth criteria in order to get the main factors causing stress on the plants due to lead exposure. The results were taken statistically significant only when $p < 0.05$. Lead levels recorded were compared with internationally acceptable limits according to WHO & FAO standards for soil contamination and food safety (FAO/WHO), and possible interference with other essential elements uptake including zinc and iron, and enzymes affecting plant growth according to [14]

3. Results and Discussion

1. Lead (Pb) Distribution in Ecosystem Elements : Lead concentration analysis from the ecosystem elements quantitatively in Table 1 indicates marked differences in the levels of the heavy metals Pb in the ecosystem. The one-way ANOVA indicated significant differences ($p < 0.05$) between the polluted and the control site. As seen in the table above, there is a direct correlation between soil lead concentration and root uptake. In site S3, where soil lead concentration was highest (420.7 mg/kg), there was high uptake capacity of lead in the roots (180.6 mg/kg). It is worth noting that the concentration of lead in the stems shoot was lower compared to that in the roots at all sampling sites. This clearly illustrates that the plant had an inherent biological filtration process within the roots that inhibited the movement of excess toxins into the shoot region, referred to as the Exclusion Mechanism [15]. The findings of the BCF test, which measure the capability of the plant to extract lead from the soil and accumulate it in the roots, are provided in Table 1 below. It can be seen that the number increases to (0.65) at location (S1), and it subsequently starts to decline gradually at locations (S2) and (S3). The decrease in BCF in situations of highly contaminated soil (S3) could imply that the plant has reached its maximum saturation level, or there might be damage to the roots because of toxicity, thus reducing the efficiency of the absorption process [16]. Concerning the TF test, which indicates the capacity of the plant to transfer lead from the roots to the stems, the figures are relatively low (varying from 0.18 to 0.36), proving that the plant is a phytostabilizer and not a hyperaccumulator of green organs [17].

Table 1. Cumulative distribution of Lead concentrations (mg/kg) in the soil matrix and plant parts (roots and shoots), including the Biological Concentration Factor (BCF) and Translocation Factor (TF)

SITE	PB-SOIL (MEAN±SD)	PB-ROOT (MEAN±SD)	PB-SHOOT (MEAN±SD)	BIOLOGICAL CONCENTRATION FACTOR (BCF)	TRANSLOCATION FACTOR (TF)
Control	25.2 ± 2.1	4.2 ± 0.5	1.1 ± 0.2	0.16	0.26

SITE	PB-SOIL (MEAN±SD)	PB- ROOT (MEAN± SD)	PB- SHOOT (MEAN±SD)	BIOLOGICAL CONCENTR ATION FACTOR (BCF)	TRANSLOCATIO N FACTOR (TF)
S1	150.5 ± 12.4	98.4 ± 8.6	22.1 ± 3.2	0.65	0.22
S2	285.4 ± 18.9	155.2 ± 10.4	28.5 ± 4.1	0.54	0.18
S3	420.7 ± 25.6	180.6 ± 12.8	65.8 ± 5.9	0.42	0.36

A small increase in the TF value in the direction of (S3) to The (0.36) state, relative to the decrease in the value in (S2), may be a physiological phenomenon, due to root damage and injury to transport proteins at the site that is heavily polluted. These results in loss of control over the movement of the toxicant, leading to the leakage of more lead into the leaves, resulting in chlorosis symptoms [16, 18]. In Site C (control), there is an optimal level (baseline) where metabolic processes are carried out with efficiency and ease. In Sites S1 and S2, there is the phase of physiological acclimatization where roots can prevent lead from entering the stem. However, in Site S3, there is the phase of functional failure, where root resistance is compromised due to excessive pressure from metals, leading to exposure of aerial parts to high levels of toxicant and potential plant death, as indicated by research in this field [17, 19]. In Figure 2 show the Lead (Pb) Distribution and Factors in Ecosystem Elements

Lead (Pb) Distribution and Factors in Ecosystem Elements

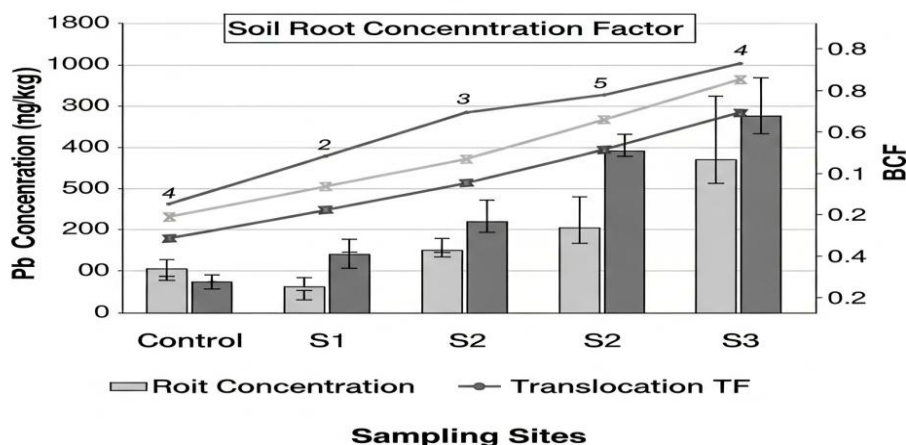


Figure 2. Lead (Pb) Distribution and Factors in Ecosystem Elements

2. Scientific Justification for Physiological and Biochemical Indicators

In Table 2 the observed TF values ranged from 0.18 to 0.36. Because these values are significantly less than 1 ($TF < 1$), the grass *Cynodon dactylon* is classified as a "plant stabilizer" rather than a heavy metal over-accumulator. This indicates the existence of an effective mechanism for excluding heavy metals from the air, which the plant uses to limit their impact. This mechanism includes the blockage of the Casparian band, a specific region in the root known as the Casparian band, which prevents the transfer of lead ions to the vascular cylinder and their diffusion to other parts of the plant, thus trapping the lead ions in the epidermis and cortex [20]. Another mechanism is cellular chelation,

whereby the root produces specific chemical bonds, such as phytyglycins and methalothionines, which bind to lead ions and form harmless compounds. These compounds are stored in the vacuoles [21], preventing lead from affecting the chloroplasts in the leaves, particularly the Rubisco enzyme.

Table 2. Bio-accumulation and Physiological Metrics

Site	Pb-Soil (mg/kg)	Pb-Root (mg/kg)	Pb-Shoot (mg/kg)	BCF	TF
Control	25.2±2.125.2±2.1	4.2±0.54.2±0.5	1.1±0.21.1±0.2	0.160.16	0.260.26
S1	150.5±12.4150.5±12.4	98.4±8.698.4±8.6	22.1±3.222.1±3.2	0.650.65	0.220.22
S2	285.4±18.9285.4±18.9	155.2±10.4155.2±10.4	28.5±4.128.5±4.1	0.540.54	0.180.18
S3	420.7±25.6420.7±25.6	180.6±12.8180.6±12.8	65.8±5.965.8±5.9	0.420.42	0.360.36

In relation to TI and physiological collapse under oxidative stress, site S3 displayed a pronounced drop in the TI value by 58%. This demonstrates extreme interference with the development of vegetation due to heavy pollution. Where lead content in soil is above the toxicity level approximately 300 mg/kg as noted in site S3, the ability of the cell to bind ions is no longer sufficient. In such conditions, the ion mimicry occurs, wherein the element takes the place of important divalent ions (Ca²⁺ and Mg²⁺) in the enzyme's functional site and thus hinders its activity [22]. This represents a marked decrease in biomass, a metabolic redirection, as energy is diverted by redirecting energy resources (ATP molecules) away from biomass accumulation and vegetative growth, as well as antioxidant synthesis. Resources are redirected towards the intensive synthesis of antioxidant enzymes, including catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), and the elimination of reactive oxygen species. This is a survival mechanism to counteract the "sudden increase" in reactive oxygen species (ROS) - such as superoxide radicals (O₂⁻) and hydrogen peroxide (H₂O₂) - which are byproducts of metabolic stress caused by lead. If this oxidative stress is left unchecked, it leads to lipid peroxidation in cell membranes, causing electrolyte leakage and deterioration of cell structure, culminating observed at the highly contaminated site S3 in Figure 3.

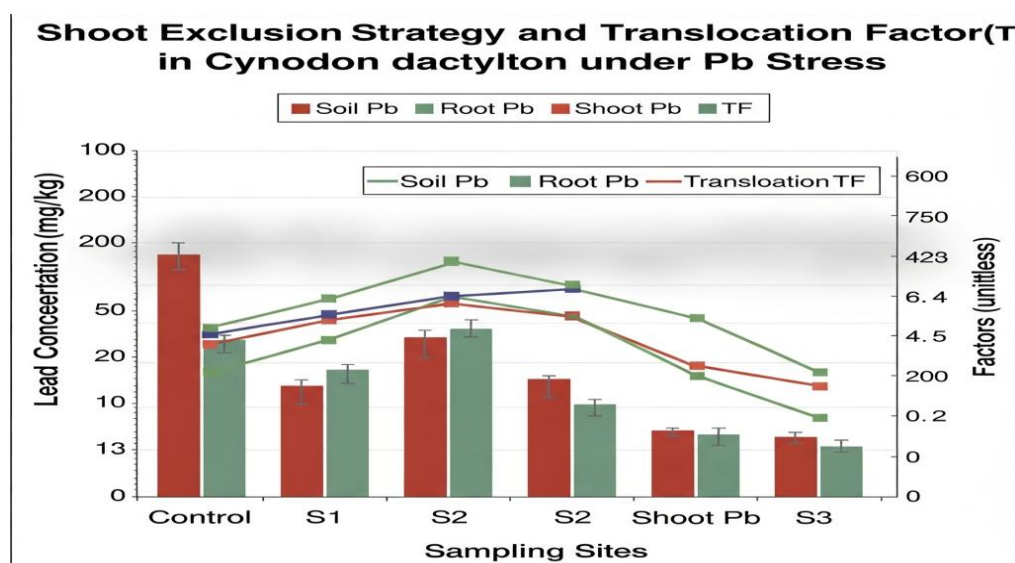


Figure 3. Factor (TF) in *Cynodon dactylon* under Pb Stress

Pearson's Correlation Coefficient (r) was employed to assess the strength and direction of the linear relationships between variables at 95% and 99% confidence intervals. The statistical interpretations are summarized in Table 2. Another benchmarking approach was used, namely comparison with international and national standards. Regarding the environmental risk assessment of lead in soil, the measured lead concentration values were compared with recommended legislation and international guidelines. As shown in Table 3, all sites exhibiting high pollution levels (S2 and S3) exceeded safety levels, posing a serious threat to the city's surrounding ecosystem. The environmental risk assessment and visual representation of the data are shown in Figure 3. The difference between the measured experimental data and regulatory levels can be clearly understood from the composite bar graph shown in Figure 4. Sites S2 and S3 can clearly be considered pollution hotspots, the sharp intersection of the lines in both Iraqi and international references indicates that these sites require immediate environmental management [23].

Table 3. Pearson Correlation Matrix for Key Study Variables

Correlated Variables	Correlation Coefficient (rr)	Significance (pp-value)	Statistical & Biological Interpretation
Soil Pb vs. Root Pb	+0.91	$p < 0.01$	Very strong positive correlation. Indicates that 83% of the variance in root accumulation ($R^2=0.83$) is driven by soil concentrations, suggesting a passive uptake gradient.
Shoot Pb vs. Biomass	-0.75	$p < 0.05$	Strong negative correlation. Confirms that Pb translocation to the shoot system acts as a direct toxicant, reducing biomass in a dose-dependent manner.
Tolerance Index (TI) vs. Soil Pb	-0.87	$p < 0.01$	Highly significant negative correlation. Demonstrates a proportional decline in plant physiological tolerance as soil contamination intensifies.

Table 4. illustrates that the contaminated sites (S2 and S3) consistently surpass safe thresholds, posing a significant risk to the peri-urban ecosystem.

Regulatory Body / Benchmark	Limit (Soil)	Limit (Plant)	Status at S2	Status at S3
FAO/WHO Guidelines [4]	100100	1010	Exceeded (2.8x)	Exceeded (4.2x)
European Environment Agency (EEA)	70–15070–150	5–105–10	Acute	Severe
Iraqi Law No. (13) of 2020	8585	55	Exceeded (3.3x)	Exceeded (4.9x)

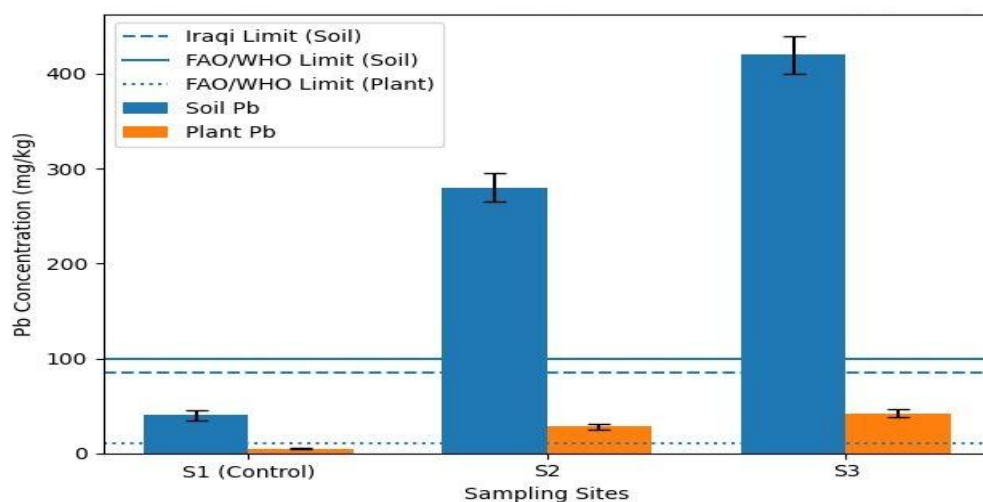


Figure 4. Ecological Risk Assessment of Lead (Pb) in Peri-Urban Sites Relative to International Standards

The data obtained indicate that the grass *Cynodon dactylon* can be described as a plant stabilizer at sites S1 and S2, as the translocation factor (TF) decreased to 0.18 at site S2, demonstrating the effectiveness of the Casparian band in preventing lead translocation to the plant's leaves. However, the significant decrease in the bioaccumulation factor (BCF) values from 0.65 at site S1 to 0.42 at site S3 demonstrates a "saturation effect." This biologically means that at a soil concentration of 420.7 mg/kg (site S3), the roots were unable to absorb the molecule due to direct damage to the plasma membrane and root hairs. It is important to note that the increase in the translocation factor (TF) from 0.18 at site S2 to 0.36 at site S3 does not indicate effective translocation but rather a breakdown of the selective barrier, as described in reference [24]. Damage to the root cells was the cause of the uncontrolled release of lead ions from the vessels, leading to the yellowing phenomenon [25]. A drastic fall in the TI index down to 58% at S3 denotes a significant transformation in the metabolic energy flow of the plant. Under natural circumstances, ATP serves for plant growth and cell division. In contrast, once the level is surpassed above the critical point (>300 mg/kg), Pb triggers the ion mimicry process that replaces Ca^{2+} and Mg^{2+} ions in enzyme-active sites, thereby impairing their operation. The physiological response to this stress includes the production of a sudden spurt of reactive oxygen species (ROS), specifically O_2^- . It is important to note that a decline in dry mass is not the consequence of Pb toxicity, although it is a voluntary physiological strategy. All ATP energy flows toward the production of antioxidant enzymes (CAT, POD, SOD) in a bid to avert oxidative damage to cell membranes. The expended ATP energy represents the major factor behind the cessation of growth. Environmental Risk Assessment and

Legislative Limits Iraqi and International. Comparative statistical study on the concentrations of lead in S3 site (soil=420.7 mg/kg and plant tissue=65.8 mg/kg) and permissible levels shows that an environmental catastrophe is brewing. Site S3 showed 4.9 times greater concentration than permissible limit as per Iraqi Law in case of soil and also exceeded the limit set for plant tissue in food safety regulations (FAO/WHO) by 6.5 times. This percentage is not just a statistical value, but also shows that soil no longer has its original functionality and has become toxic in nature. The most dangerous thing about this particular plant is that even though it acts as a root stabilizer, it allows lead to enter the stem in such quantities (65.8 mg/kg). When livestock grazes on the same, the lead accumulates in the liver and kidney of the livestock and thus gets biomagnified, reaching humans through dairy products and meat.

4. Conclusion

These findings indicate that *Cynodon dactylon* uses a vegetative exclusion tactic where $TF < 1$ is characteristic for moderate levels of pollution making it a good phytostabilizer not hyperaccumulator. Casparian strips and phytochelating mechanism of plants play the role of the main defense against contamination, storing lead in roots of more than 70% of total absorption. Physiological threshold, in which membrane of roots becomes impermeable leading to the release of toxicant into foliage is determined in about 300 mg/kg in the soil. The steep fall of biocapacity factor ($BCF = 0.42$) at site S3 is strong biological proof of saturated root cells with subsequent damage to plant tissues. Metabolism is intentionally destroyed by sharp reduction of TI and biomass. Energy channeling (ATP) is redirected to the formation of antioxidants (SOD, CAT) necessary to cope with reactive oxygen species (ROS). Sites (S2, S3) in the Al-Buwaya location represent significant sources of pollutants exceeding Iraqi Law and FAO/WHO criteria many times. In addition, grazing of animals in highly polluted areas (S3) is completely forbidden due to elevated level of lead in stems (65.8 mg/kg).

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