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ORIGINAL ARTICLE

Effect of Silver Nanoparticles and $Pb(NO_3)_2$ on the Yield and Chemical Composition of Mung bean(*Vigna radiata*)

Saeideh Najafi, Rashid Jamei*

Department of Biology, Faculty of Science, Urmia University, Iran

Phone: 09141464357

*E-Mail: r.jamei@urmia.ac.ir

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Phytotoxic effects of Pb as $Pb(NO_3)_2$ and silver nanoparticles on Mung bean (*Vigna radiata*) planted on contaminated soil was assessed in terms of growth, yield, chlorophyll pigments, phenol and flavonoid content at 120 ppm concentration. Experiments were carried out with 4 treatments in 10 days. Treatments were including (T1) control, (T2) silver nanoparticles (50 ppm), (T3) Pb as Pb $(NO_3)_2$ (120 ppm) and (T4) silver nanoparticles (50 ppm) plus Pb as $Pb(NO_3)_2$ (120 ppm). Regarding the pigment content, silver nanoparticles-treated plants showed a remarkable increase of chlorophyll. The loss of chlorophyll content was associated with disturbance in photosynthetic capacity which ultimately results in the reduction of *Vigna radiate* growth. Pb caused a fall in the total content of phenols, while the content of flavonoid not significantly changed. The minimum decrease in root length, weight of root fresh and stem fresh was observed in T4 group, but this factors increased in the other treatments. Also, length of stem and seedling height decreased in control group. Increase length and fresh weight of stem in Pb-treated plants suggest that compatible solutes may contribute to osmotic adjustment at the cellular level and enzyme protection stabilizing the structure of macromolecules and organelles.

Key words: Biochemical parameters, Chlorophyll pigments, Growth, Lead pollution, Vigna radiata

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Nanotechnology has a significant effect in agriculture and main areas of the food industry. Engineered nano materials have received a particular attention for their positive impact in improving many sectors of economy and trade, including consumer products, loom, pharmaceutics, cosmetics, transportation, energy and agriculture etc., and are being increasingly produced for a Wide range of applications within industry (Novack and Bucheli, 2007, Roco, 2003). The organisms and especially those that interact strongly with their immediate environments are expected to be affected as a result to their exposition to silver nanoparticles. The effect of some nanoparticles such as silver (Choi *et al*., 2008, Sahu *et al*., 2012), nanoceria (Lopez-Moreno *et al*., 2010), TiO_2 (Ghosh *et al*., 2010, Ge *et al*., 2012), ZnO (Lopez-Moreno *et al*., 2010, Ge *et al*., 2011, Yin *et al*., 2011), copper (Lee *et al.*, 2008), etc, on plant and microbes reported in literature. Nanoparticles (nano-scal particles=NSPs) are atomic or molecular aggregates that its size is about 100 nm down to about 1 nm (Ball, 2002, Roco, 2003), that can drastically modify their physics and chemical properties compared to the bulk material. Engineered nano materials have received a particular attention for their positive impact in improving many sectors of economy and trade, including consumer products, loom, pharmaceutics, cosmetics, transportation, energy and agriculture etc., and are being increasingly produced for a wide range of applications within industry (Novack and Bucheli, 2007, Roco, 2003). Heavy metals provide a contaminated environment and dangerous for human, plants and other biota. Presence of heavy metals in soil may be naturally occurring or due to human activities such as metallic industries, contaminated fertilizers, herbicides or insecticide and irrigation with contaminated ground water (Duruibe *et al*., 2007). These metals adversely effects on plant production, leading to disruption of vital biochemical and ecological process (Nriagu and Nieboer, 1988, Bitton and Dutka, 1986). Heavy metal induced oxidative stress that these results reported in the literature (Leonard *et al*., 2004). Some these metals have bio-importance as trace and non-biodegradable elements but, the bio toxic effects of many of them in plants biochemistry are of great concern. Heavy metals include metals such as aluminum, zinc, Pb (lead), cadmium, chromium, copper, nickel and manganese (Phipps, 1981, Horsfall and Spiff, 2004).They are hazardous to human health and environment, through their accumulation in the soil and drinking water (Huang *et al*., 2007). Heavy metals enter in agricultural land and food chain that they affect on aquatic organisms, plant growth, animals and human health (Thornton, 1991). Lead (Pb) is one of the most widespread heavy metal contaminant and distributed trace metals in soils. Pb has no biological function but can cause morphological, physiological and biochemical dysfunctions in plants. It is a heavy metal of human and industrial activities origin (Sharma and Dubeg, 2005). The mung is the seed of *Vigna radiate*. It is mainly cultivated in India, Thailand, Philippines, Indonesia, Burma and etc. the aim of this study was to investigate the effect of silver nanoparticles and $Pb(NO_3)_2$ on the yield and chemical composition of Mung bean (*Vigna radiata*).

MATERIALS AND METHODS

Seed pretreatment with silver nanoparticles

Silver nanoparticles were prepared by means of the biological reduction of metal salt precursor (silver nitrate, $AgNO_3$) in water with aqueous extract of manna of hedysarum plant in the presence of extract of soap-root plant as a stabilizer (Forough and Farhadi, 2010). Briefly, 10 ml of freshly prepared extract of soap-root plant as a stabilizer agent was added to 100 ml of 3 mM aqueous silver nitrate solution and incubated in a rotary shaker for 2 h in dark conditions at 25 ^oC, and then 15 ml of the aqueous extract of manna of hedysarum plant as a reducing agent was added into the mixture at 86 ^oC. The mixture obtained, was purified by repeated centrifugation at 12,000 g for 20 min to obtain the fresh biologically Ag nanoparticles solution.

Seed germination and seedling development

Magnetic field pretreated and control seeds were surface sterilized with 1% NaOCl (w/v) for 5 min, washed thoroughly 3 times with distilled water and then propagated in pots containing soil and sand mixture (1:2). The pots were maintained under natural photoperiod with 35% (w/w) soil moisture content. Seed germination observed at 7th day, and germination seedlings were uprooted and measured the length, fresh and dry weight of 10 days for both control and treated seedlings.

Pigment contents (chlorophyll a, chlorophyll b and carotenoid)

The photosynthetic pigments e.g., Chlorophyll a, b and Carotenoid were extracted in 5 ml of chilled 80% acetone by grinding the leaves of salt treated seedlings in a chilled mortar and pestle. The homogenate was centrifuged at 3000 g for 10 min at 4 ^{o}C. The absorbance of the resulting supernatant was taken at 480, 645 and 663 nm. Different pigments were estimated using the following formula by Barnes as given below:

Chl a (mg/l) = 12.7 (A_{663}) – 2.69 (A_{645})

Chl b (mg/l) = 22.9 (A_{645}) –4.68 (A_{663})

Car (mg/l) =1000A_{480} –1.8Chl a - 85.02Chl b/198

The pigment concentration was calculated in g/g FW of sample and expressed as percent change (Barnes *et al*., 1992)

Total phenol

Total phenol was determined spectrophotometerically using Folin–Ciocalteu's reagent as described by Bonilla *et al*. (2003). Briefly, 4 g fresh *Vigna radiata* (the seed discarded) were ground in liquid nitrogen. A sample was then extracted in 2% HCl in methanol for 24 h in the dark and at room temperature. After centrifugation at 12,000 g for 20 min at 4 ^{o}C, the supernatant was diluted with the same extract solvent at a suitable concentration for assaying total phenol. Two hundred microliters of diluted extraction was introduced into a 5.0 ml test tube. One milliliters of Folin–Ciocalteu reagent and 0.8 ml sodium carbonate (7.5%) were then added and the contents mixed and allowed to stand for 30 min. Absorption at 765 nm was measured in a Bio wave UV–Vis spectrophotometer (English production).Total phenol content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample using a standard curve generated with 50, 100, 150, 200, 250, 300, 350, 400, and 500 mg/l of gallic acid (Bonilla *et al*., 2003).

Determination of flavonoid content

The flavonoid contents of the extracts were determined by the colorimetric method with some modifications (Jerman *et al*., 1989). The *Vigna radiata* extract (0.1 ml) was mixed with 1.25 ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 150 µl of a 10% $AlCl_3.H_2O$ solution was added. After 6 min, 500 µl of 1 M NaOH and 275 µl of distilled water were added to the mixture. The solution was mixed well and the intensity of the pink color was measured at 510 nm. The results were expressed as milligrams of catechin equivalents per gram of sample (mg CEs/g extract).

Statistical analysis

The data obtained from the experiments were analyzed and calculated. As the experimental design is completely randomized design and data for each experiment were analyzed by one-way ANOVA with factorial arrangement to determine the effects of magnetic treatment. Means were compared using Duncan's multiple-range test at a 5% level of significance by SPSS software version 16.

RESULTS AND DISCUSSION

Lead (Pb) exerts a negative effect on morphology, growth and photosynthesis processes of plants. Lead inhibited seed germination of *Spartiana alterniflora* (Morzck and Funicclli, 1982). Inhibition of growth may be due to the interference of lead with important enzymes. Lead inhibited early seadling growth in barley (Stibotova *et al*., 1987), tomato, egg plant (Khan and Khan, 1983) and certain legumes (Sudhakar *et al*., 1992). Also, lead inhibited root and stem elongation and leaf expansion in *Allium* species (Gruenhange and Jager, 1985), barley (Juwarkar and Shende, 1986) and *Raphanus sativus*. Inhibition of root elongation depends on concentration of lead and ionic composition (Matecka *et al*., 2008).

Chlorophyll a, b and carotenoid content increased with Pb treatment, while silver nanoparticles had a negative relationship with photosynthesis pigments (Fig 2). Chlorophyll a and b helps in photosynthesis by absorbing light energy and they are very sensitive to environmental stresses such as heavy metals (Ekmekci *et al*., 2008). As shown in Fig 2, the chlorophyll a and b significantly decreased in Pb-treated plants. Similar results were obtained by other researchers (Wu *et al*., 2003, Wang *et al*., 2009, Zengin and Munzuroglu, 2005). Decrease in chlorophyll content may be due to replacement of Mg with heavy metals in chlorophyll structure (Kupper *et al*., 1998), reduce synthesis chlorophyll due to inhibition of enzymes activity such as δ-aminolevulinic acid dehydratase (ALA dehydratase) (Padmaja *et al*., 1990) and protochlorophyllidereductase (Van Assche and Clijsters, 1990), decrease in density, size and the synthesis of chlorophyll and inhibition in the activity of some enzymes of Calvine cycle(Baryla *et al*., 2001, Benavides *et al*., 2005). In soils, the mobility of silver nanoparticles in pore water is an essential condition for interactions with plant roots. The silver nanoparticles were located in the nucleus and applying the definition of McGrath and Zhao in 2003, the *Medicago sativa* and *Brassica juncea* were hypercumulators of silver (Rucuciu and Creanga, 2007).

Based upon these results it can be stated that, generally, Pb cause a fall in the total content of phenols, while the content of flavonoid not significantly changed (Fig 3). Compared to this study (Kaimoyo *et al*., 2008, Dannehl *et al*., 2011) have found that sub-lethal levels of electric current can be used to induce plant defence reactions and activity as an abiotic elicitor to enhance the secondary metabolite production in fenugreek, chickpea roots, and tomatoes.

Content of phenol and flavonoid increased in Pb treatment but these factors decreased in the other groups. Phenolics have different functions in plants. Phenylopropanoid metabolism and the amount of phenolic compounds can be increased under various environmental factors and stress conditions (Diaz *et al*., 2001, Sakihama and Yamasaki, 2002, Grace and Logan, 2000, Lavola *et al*., 2000). The synthesis of flavonoids is induced when plants are in low temperature and low nutrient condition (Sakihama and Yamasaki, 2002, Ruiz *et al*., 2003). Heavy metals influenced on phenylpropanoid metabolism, flavonoid and phenol (Michalak, 2006). Morgan *et al*. (1997) reported that general chelating ability of phenolic compounds is probably related to the aromatic rings and high nucleophilic character rather than to specific chelating groups within the molecule.In addition, the flavonoids have been implicated in tolerance to stressors such as UV-B, drought and heavy metals (Gould, 2004). Harris and Bali (2008) reported the limits of uptake and the distribution of silver nanoparticles in *Brassica juncea* and *Medicago sativa* (Harris and Bali, 2008).

The results showed that Pb caused necrosis in leaf. The silver nanoparticles improved necrosis in plant leaf (Fig 4). At high concentration, Pb become toxic, causing symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth (Marschner, 1995). Salt *et al.* (1995) observed a correspondence between Cd distribution and chlorosis or necrosis in leaves of *Brassica juncea.*

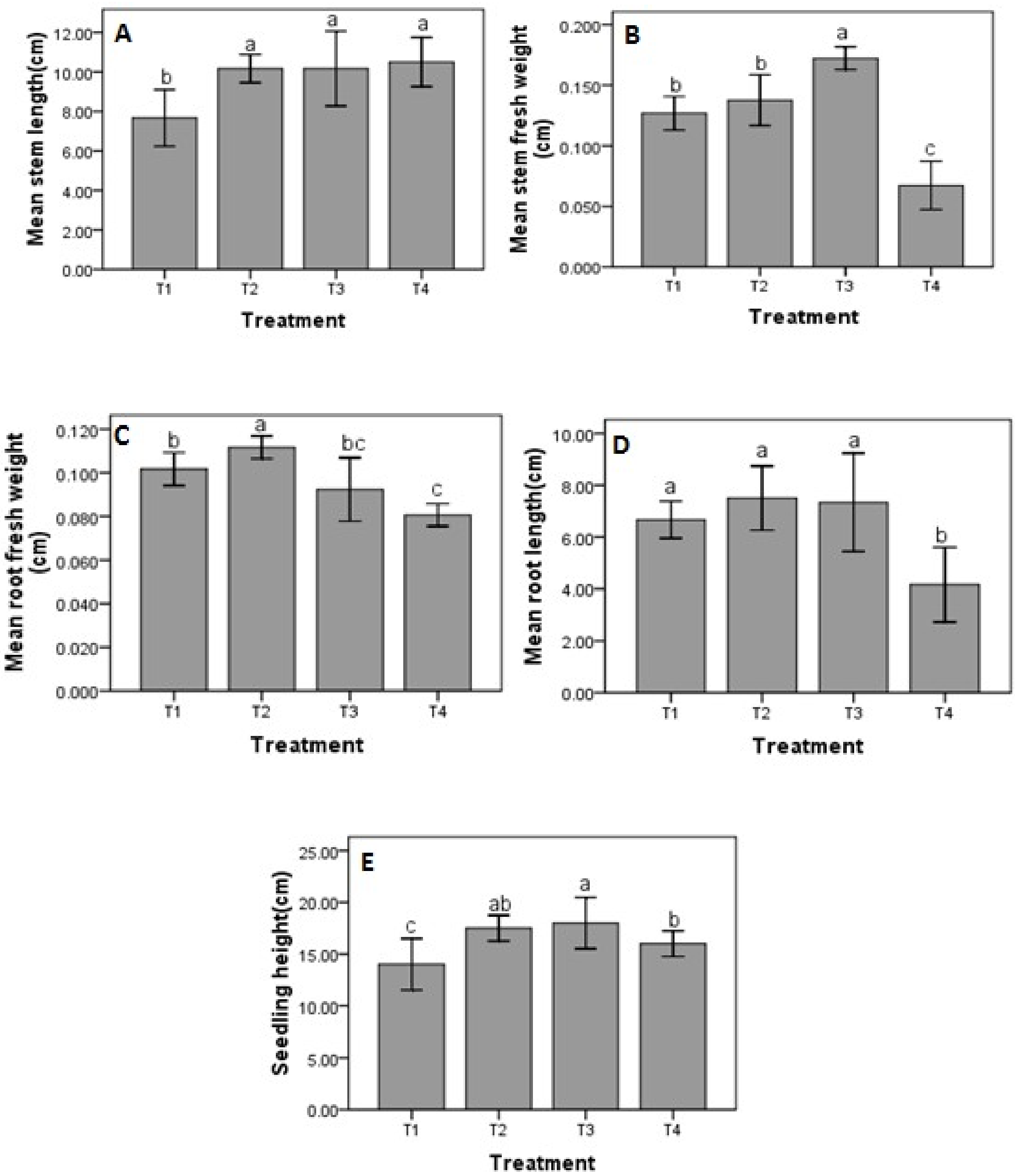

Figure 1. Influence of Pb as Pb(NO$_3$)$_2$ and silver nanoparticles on seedling height, length of root and stem,fresh weight in root and stem of *Vigna radiata.* Bars represent means ± standard error. Means followed by the same letter are not significantly different (P<0.05) as determined by Duncan's multiple-range test. (T1) control, (T2) silver nanoparticles (50 ppm), (T3) Pb as Pb(NO$_3$)$_2$ (120 ppm) and (T4) silver nanoparticles (50 ppm) plus Pb as Pb(NO$_3$)$_2$ (120 ppm).

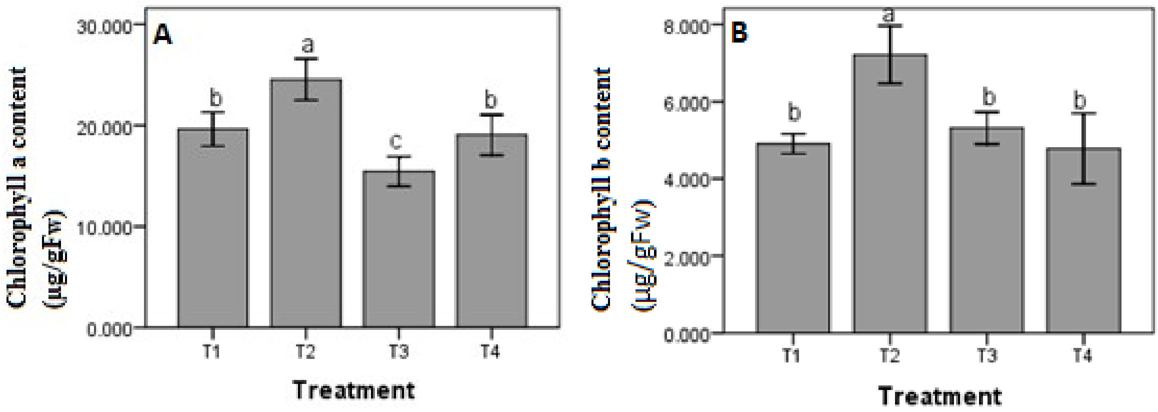

Figure 2. Influence of Pb as $Pb(NO_3)_2$ and silver nanoparticles on Chlorophyll a and b content of *Vigna radiata.* Bars represent means ± standard error. Means followed by the same letter are not significantly different (*P*<0.05) as determined by Duncan's multiple-range test. (T1) control, (T2) silver nanoparticles (50 ppm), (T3) Pb as $Pb(NO_3)_2$ (120 ppm) and (T4) silver nanoparticles (50 ppm) plus Pb as $Pb(NO_3)_2$ (120 ppm).

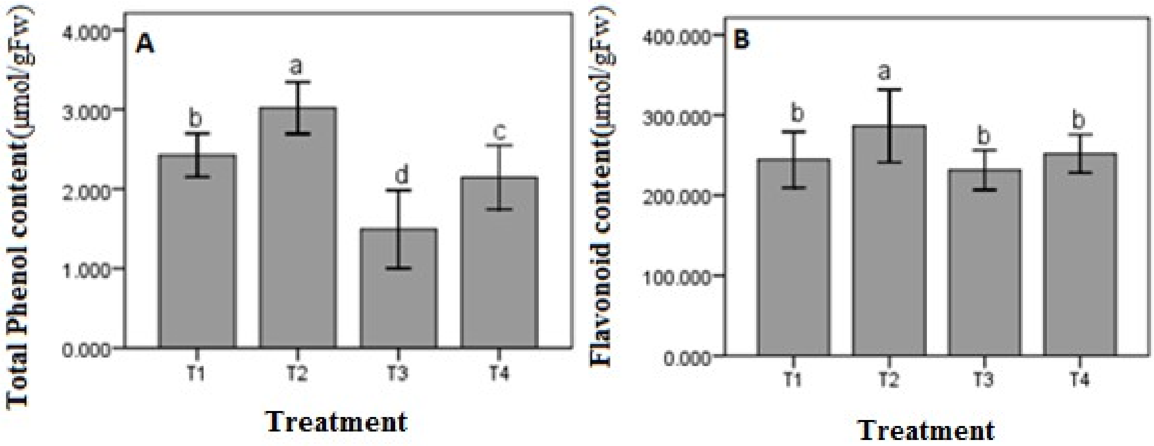

Figure 3. Influence of Pb as $Pb(NO_3)_2$ and silver nanoparticles on phenol and flavonoid content of *Vigna radiata.* Bars represent means ± standard error. Means followed by the same letter are not significantly different (*P*<0.05) as determined by Duncan's multiple-range test. (T1) control, (T2) silver nanoparticles (50 ppm), (T3) Pb as $Pb(NO_3)_2$ (120 ppm) and (T4) silver nanoparticles (50 ppm) plus Pb as $Pb(NO_3)_2$ (120 ppm).

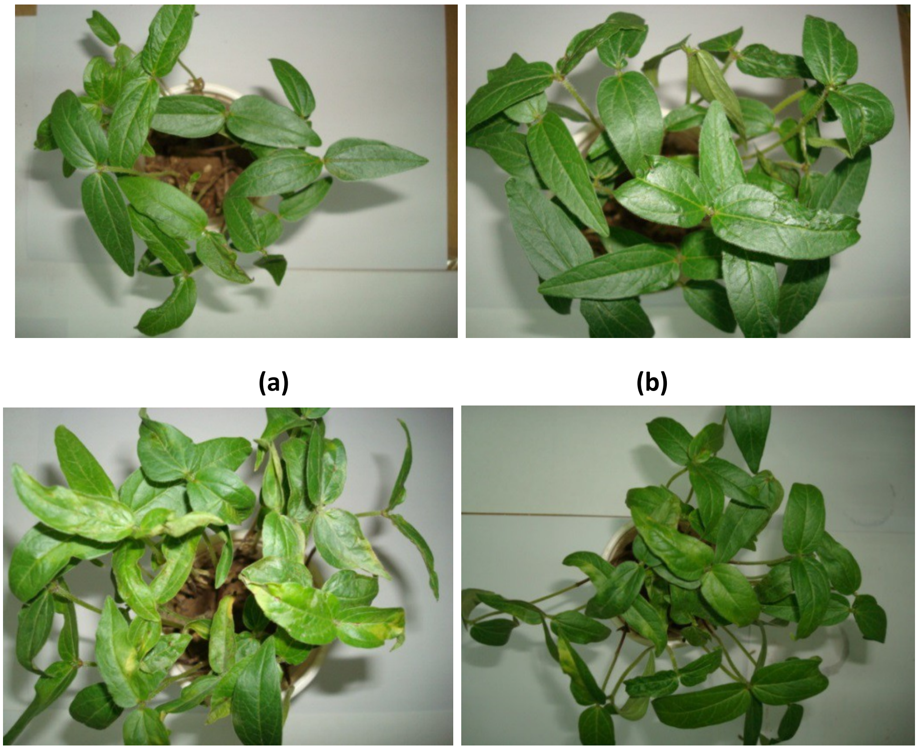

Figure 4. Influence of Pb as $Pb(NO_3)_2$ and silver nanoparticles on leaf states in *Vigna radiate*. (a) T1, (b) T2, (c) T3 plus (d) T4.

CONCLUSION:

The obtained results indicated that silver nanoparticles and Pb as $Pb(NO_3)_2$ have different effects on the yield and chemical composition of Mung bean (*Vigna radiata*). Generally, it can be concluded that the applied silver nanoparticles treatments improved the necrosis created in plant leaf.

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