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THE SYNERGISTIC INFLUENCE OF BIOFERTILIZATION AND COMPOSTING ON BIO-REMEDIATING CADMIUM POLLUTED SOIL CULTIVATED WITH MAIZE PLANTS

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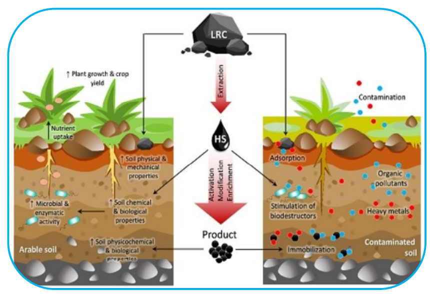
ABSTRACT



A pot experiment was conducted in the green house of King Abdulaziz University to investigate the synergistic influence of compost and bio-fertilizer on bioremediation of cadmium ($CdCl_2$) in a soil cultivated with maize plants. A few soil cellulose-decomposing microorganisms was isolated; the highly efficient microbial strain in cellulose degradation (4.9 cm clear zone on CMC agar medium) was identified as Bacillus cereus. Plant residues were aerobically composted till maturity stage (60-day-old) and had N-content, 2.8%; K-content, 2.5%; P-content, 0.35% and C:N ratio, 13). The dry weight of maize plants treated with 0.1% $CdCl_2$ reached 0.66 g/plant meanwhile increasing the rate of $CdCl_2$ to 0.5% was highly toxic and completely prevented maize plants to grow. Biofertilization with Bacillus cereus as such produced the highest maize dry weight (8.66 g/plant) while the control treatment yielded 7.33 g/plant; the increase over control reached 18.15%. Inoculation of maize plants with B. cereus lowered the toxic effect of $CdCl_2$ and improved growth of maize plants where biofertilization did increase dry weight over $CdCl_2$ (1%) treatment with 51.52%. Treatment of $CdCl_2$ 0.1% induced nitrogen content reached 1.5 mg/g plant, inclusion of B. cereus to 0.1% $CdCl_2$ treatment significantly augmented nitrogen content of maize plants; the percentage of increase reached 26.67. B. cereus treatment produced the highest significant increase (0.59 mg/g) in comparison with the rest treatments; the increases over control, $CdCl_2$ 0.1% and $CdCl_2$ 0.1% + B. cereus were 22.92%, 96.67% and 68.57% consecutively. For phosphorus content of maize plants, inoculation with B. cereus produced the highest significant increase (0.59 mg/g) in comparison with the rest treatments; the increases over control, $CdCl_2$ 0.1% and $CdCl_2$ 0.1% + B. cereus were 22.92%, 96.67% and 68.57% consecutively. Regarding compost application, the highest significant increase in maize dry weight was obtained due to the application of compost at the rate of 3%. Application of compost together with $CdCl_2$ 0.1% augmented maize plant dry weight with 203.03 and 404.55 % over treatment of $CdCl_2$ 0.1% as such. Compost at the rate of 3% recorded the highest N-content (4.4 mg/g) followed by compost 1% treatment that produced 3.3mg/g N-content. Biofertilization with B. cereus in presence of both compost (1%) and $CdCl_2$ (0.1%) produced dry weight reached 2.33 g/plant; the increasing percent over 0.1% $CdCl_2$ treatment as such reached 253.03. Increasing compost rate to 3% (0.1% $CdCl_2$ + B. cereus + compost 3%) raised the increasing percent to 556.06. For maize plants nitrogen content (mg/g), B. cereus in presence of compost

(1%) and $CdCl_2$ (0.1%) significantly increased N-content, the increase reached 66.67% in comparison with $CdCl_2$ (0.1%) treatment as such. Additional increase in N-content of maize plants was obtained when the compost dose of 1% was replaced by 3% at the same previous treatment; the percentage of increase reached 93.33. As for P-content of maize plants, it ranged from 0.30 mg/g to 0.71 mg/g being the lowest due to the application of 0.1 $CdCl_2$ and the highest owing to the treatment of 3% compost. Application of both B. cereus and compost (1%) to maize plants treated with 0.1% $CdCl_2$ significantly improved P-content where the increase reached 33.33%. Additional significant increase was recorded when the compost dose was elevated to 3% where the increase reached 56.67% in comparison with $CdCl_2$ (0.1%) as such. P-content of maize plants ranged from 5.18 mg/g to 9.61 mg/g being the lowest with the treatment of $CdCl_2$ (0.1%) and the highest due to the treatment of $CdCl_2$ 0.1% + B. cereus + compost 3%. Combination of B. cereus and compost (1%) together with $CdCl_2$ (0.1%) lowered the toxic effect of $CdCl_2$ on maize plants where this treatment significantly increased K- content with 21.24%. Raising the compost dose to 3% did more increase in K- content of maize plants to reach 47.49%.

KEY WORDS: *Bioremediation, biofertilization, compost, Cadmium chloride, maize plants.*

INTRODUCTION

Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those most commonly found at contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and nickel (Ni) (GWRTAC, 1997). Soils are the major sink for heavy metals released into the environment by anthropogenic activities and unlike organic contaminants which are oxidized to carbon oxide by microbial action; most metals do not undergo microbial or chemical degradation (Kirpichtchikova *et al.*, 2006) and their total concentration in soils persists for a long time after their introduction (Adriano, 2003). Bioremediation is a biological process for cleaning up of pollutants from the environment. Heavy metals have recently received great attention of researchers; mainly because they cannot be degraded or destroyed and they enter our bodies via food, drinking water and air (Adarsh *et al.*, 2007). Gomaa *et al.* (2012) stated that application of heavy metals (Cd & Zn) negatively affected wheat plants growth (shoot dry weight); while inclusion of biofertilizers (*Rhizobium* and *Azospirillum*) significantly increased shoot dry weight in the presence of 300ppm Cd in comparison with the positive control (100% NPK). Furthermore, they stated that the bio-remediating bacteria, i.e. *Rhizobium* and *Azospirillum* neutralized the harmful impact of heavy metals on wheat plants growth. Application of *Alcaligenes faecalis* rhizobacterium in Cd-polluted soil and cultivated with faba bean improved plant growth in the presence of 5000 ppm or 7500 ppm $CdSO_4$; the percentages of increases in plant fresh weights reached 58.18% and 56.53% over the same but not inoculated Cd concentrations treatments (Gomaa *et al.*, 2016).

A lack of information are present regarding application of compost in bio-remediation of heavy metals; Mahmoud and Abd El-Kader (2014) revealed that application of phosphogypsum either alone or in combination with compost reduces uptake of heavy metals (Pb, Cd and Zn) in contaminated soil cultivated with canola plant.

So, the aim of this research work is to figure out the possible role of applying compost and/or bio-agent in alleviating the harmful effect of heavy metals (Cd & Pb) on maize plants grown in sandy soil.

MATERIALS AND METHODS

Isolation of cellulose-decomposing microbial strains:

A number of cellulose-degrading microbial cultures was isolated from the rhizosphere soil of different plants, *viz* lettus (*Lactuca sativa)*, rubber tree (*Calotropis procera)*, Prosopis or acacia (*Acacia* sp.), aloe vera (*Aloe barbadensis*) plants in a addition to pigeons waste and previously made mature compost. The active microbial isolates were obtained from tubes that gave positive result (cellulosic filter paper degradation - Plate 1). The obtained isolates were purified by streaking on CMC agar plates, incubated at 30°C for 3-5 days. The pure isolated colonies were maintained on CMC agar slants at 4°C for further analysis.

Screening the isolated microorganisms for cellulose degradation:

For determining the relative activity of the different isolated cultures in cellulase production, the disc diffusion method was applied according to Bauer *et al*. (1966) where the agar plates were prepared with 0.5% CMC and 2.0% agar using Congo red stain.

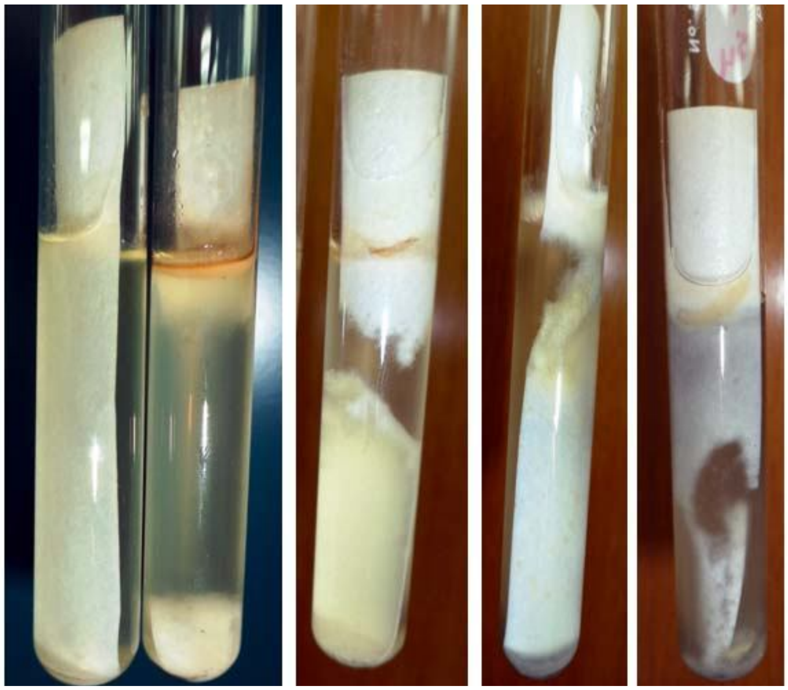


Plate 1: Various cultures of cellulose-decomposing microorganisms showing different degrees of cellulose papers degradation.

Identification of the isolated microorganisms:

The highly efficient microbial strain in cellulose degradation was identified at Macrogen Inc., Seoul, South Korea by using 16S rRNA Sequencing.

Aerobic composting of plant residues:

The plant residues were aerobically composted according to Motwali *et al.* (2016) and Qattan *et al.* (2017).

Experimental treatments:

A pot experiment was conducted in the green house of King Abdulaziz University to investigate the synergistic influence of compost and cellulose - decomposing bacterium on bioremediation of cadmium in a soil cultivated with maize plants. Plastic pots of 23 cm diameter and 21depth were filled with 8 kg soil per each. The following treatments were taken into consideration:

- Control.
- Inoculation with *B. cereus*.
- Cadmium ($CdCl_2$) 0.1%.
- Cadmium 0.5%.
- Cadmium 0. 1% + Inoculation with *B. cereus*.
- Cadmium 0.5% + Inoculation with *B. cereus*.

- Compost 1%.
- Compost 3%.
- Cadmium 0.1% + Compost 1%.
- Cadmium 0.1% + Compost 3%.
- Cadmium 0.5% + Compost 1%.
- Cadmium 0.5% + Compost 3%.
- Cadmium 0.1% + *B. cereus* + Compost 1%.
- Cadmium 0.1% + *B. cereus* + Compost 3%.
- Cadmium 0.5% + *B. cereus* + Compost 1%.
- Cadmium 0.5% + *B. cereus* + Compost 3%.

RESULTS AND DISCUSSION

The isolated microbial strain:

One microbial isolate that isolated from previously made compost (Motwali *et al.*, 2016) and show the highest efficiency in cellulose degradation (4.9 cm clear zone on CMC agar medium - Plate 2) was identified by Macrogen Inc., Seoul, South Korea by using 16S rRNA Sequencing as *Bacillus cereus*.

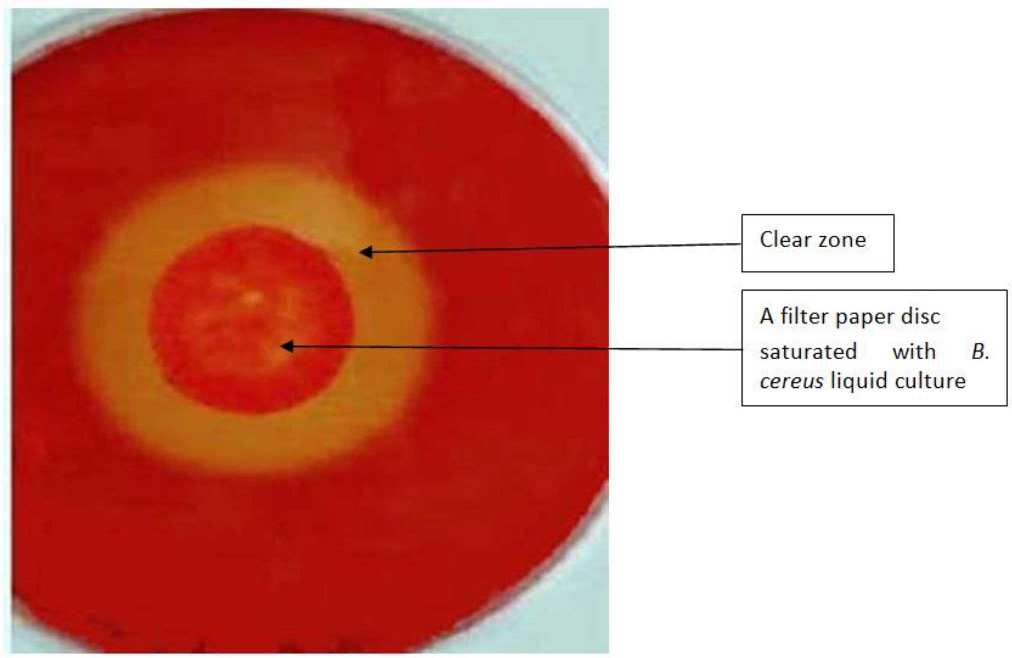


Plate 2: A clear zone of cellulose decomposition on CMC agar medium by *B. cereus* after incubation for five days at 30°C.

The composted plant materials:

After 60 days of composting, the plant materials reached their maturity and had the following characteristics: moisture content (%), 30; pH, 8.9; Organic matter (%), 62; N-content (%), 2.8; K-content (%), 2.5; P- content (%), 0.35; Cd content (ppm), < 0.5 and C/N ration, 13 (Qattan *et al.*, 2017).

Influence of various treatments on bioremediation of Cd and growth of maize plants:

Table (1) shows the impact of inoculation with *Bacillus cereus* in presence or absence of cadmium chloride ($CdCl_2$) at both concentrations 0.1% and 0.5% on dry weight (g/plant) and macro nutrients (N, P and K) as mg/g dry weight of maize plants.

Concerning maize dry weight, Biofertilization with *Bacillus cereus* as such produced the highest maize dry weight (8.66 g/plant) while the control treatment yielded 7.33 g/plant; the increase over control treatment reached 18.15%. This finding was found in agreement with that obtained by El-Kholy and Gomaa (2000) who stated that biofertilization with *Azospirillum* and *Azotobacter* increased millet green yield in comparison with the non-biofertilized control. Also, Gaballah and Gomaa (2004) found that biofertilization of some faba bean varieties with yeast (*Rhodotorula glutinis*) augmented plant dry weight for all tested varieties in comparison with the uninoculated control. The results within hand are on the same line with those obtained by Malekzadeh *et al.* (2012) who stated that in a pot experiment on maize plants, inoculation with *Bacillus mycoides* either alone or in combination with *Micrococcus roseus* significantly increased root and shoot dry weights in comparison with the control. Moreover, Yachana *et al.* (2017) stated that maize plants inoculated with *B. megaterium* and *P. aeruginosa* showed 5% greater dry weight as compared to non-inoculated control.

The dry weight of maize plants treated with 0.1% $CdCl_2$ reached 0.66 g/plant meanwhile increasing the rate of $CdCl_2$ to 0.5% was highly toxic and completely prevented maize plants to grow. Malekzadeh *et al.* (2012) pointed out that increasing Cd levels significantly decreased maize plant root and shoot dry weights. Application of biofertilizer (*B. cereus*) together with 0.1% $CdCl_2$ increased maize plants dry weight in comparison with the application of $CdCl_2$ 0.1% as such; this increase reached 51.52 %. In another words, inoculation of maize plants with *B. cereus* lowered the toxic effect of $CdCl_2$ and improved the growth of maize plants. This is due to immobilization of cadmium by the bio-agent *B. cereus* where Gomaa *et al.* (2016) proved that *Proteus mirabilis* was able to immobilize 30.3% of the total cadmium (as cadmium sulphate – $CdSO_4.8H_2O$) from the liquid medium. Gomaa *et al.* (2012) in a pot experiment on wheat plants demonstrated that biofertilization with *Azospirillum lipoferum* and/or *Rhizobium leguminsarum* bv. trifolii in the presence of 300 ppm of cadmium sulphate ($CdSO_4$) significantly increased shoot dry weight in comparison with the positive control. Moreover, Gomaa *et al.* (2016) found that biofertilization of faba bean plants grown in a soil artificially contaminated with 5000 ppm $CdSO_4$ with *Alcaligenes faecalis* significantly increased plant height by 67.94% over the same un-inoculated treatment. In addition, they also stated that application of *Alcaligenes faecalis* augmented fresh weight of faba bean plants grown under either 5000 or 7500 ppm $CdSO_4$, the increases reached 58.18 and 56.53 % over the same non-biofertilized treatments.

Table 1: Impact of $CdCl_2$ and inoculation with *B. Cereus* on dry weight and macro-nutrients content of maize plants.

Parameters / Treatments	Dry weight (g/plant)	N-content (mg/g)	P-content (mg/g)	K-content (mg/g)
Control	7.33	2.60	0.48	5.75
Bacillus cereus	8.66	3.10	0.59	6.90
Cadmium 0.1%	0.66	1.50	0.30	5.18
Cadmium 0.5%	0.00	0.00	0.00	0.00
Cadmium 0.1% + *Bacillus cereus*	1.00	1.90	0.35	5.68
Cadmium 0.5% + *Bacillus cereus*	0.00	0.00	0.00	0.00
LSD (0.05)	1.83	0.025	0.008	0.027

Regarding the nitrogen content (mg/g) of maize plants, Table (1) also indicates that biofertilization of maize plants with *B. cereus* induced the highest significant increase when compared with the other treatments. Gomaa (2000) revealed that highly significant differences were obtained due to biofertilizer application for N_2-uptake of mungbean plant, seed and shell in comparison with the control. Treatment of $CdCl_2$ 0.1% induced nitrogen content reached 1.5 mg/g, inclusion of *B. cereus* to 0.1% $CdCl_2$ treatment significantly augmented nitrogen content of maize plants in comparison with $CdCl_2$ 0.1 % treatment as such; the percentage of increase reached 26.67.

The same trend was recorded with phosphorus content of maize plants, where inoculation with *B. cereus* produced the highest significant increase (0.59 mg/g) in comparison with the rest treatments;

the increases over control, $CdCl_2$ 0.1% and $CdCl_2$ 0.1% + *B. cereus* were 22.92%, 96.67% and 68.57% consecutively. Furthermore, $CdCl_2$ 0.1% treatment induced P- content reached 0.30 mg/g; while P-content significantly increased when the same treatment of $CdCl_2$ 0.1% was combined with the bio-fertilizer *B. cereus* to record 0.35 mg/g.

For potassium content, inoculation of maize plants *with B. cereus* yielded the highest significant value of K-content (6.90 mg/g) when compared to the other tested treatments. The combined application of $CdCl_2$ 0.1% and inoculation with *B. cereus* significantly surpassed the treatment of $CdCl_2$ 0.1% as such, the percentage of increase reached 9.7.

The influence of compost application to maize plants on mitigating the negative effect of $CdCl_2$ was illustrated in Table (2). The highest significant increase in maize dry weight was obtained due to the application of compost at the rate of 3%. The percentage of increases reached 1566.67, 17.9, 450 and 230 over the treatments of $CdCl_2$ 0.1%, compost 1%, $CdCl_2$ 0.1% + compost 1% and $CdCl_2$ 0.1% + compost 3% consecutively. Application of compost together with $CdCl_2$ 0.1% augmented maize plant dry weight with 203.03 and 404.55 % over treatment of $CdCl_2$ 0.1% as such. This means that application of compost relieves the harmful impact of $CdCl_2$ on maize plants growth.

With regard to maize plants nitrogen content (mg/g dry weight), Table (2) also indicates that use of compost at the rate of 3% recorded the highest N- content (4.4 mg/g) followed by treatment of compost 1% that produced 3.3mg/g N-content. Application of $CdCl_2$ 0.1% reduced N-content by 42.31% in comparison with the control treatment while addition of compost (1%) to the treatment of $CdCl_2$ 0.1% improved maize plants. N-content to produce 2.1 mg/g; elevating the compost dose to 3% did additional increase in N-content that reached 33.3% over treatment of $CdCl_2$ 0.1% + compost 1%.

Concerning maize plants P-content (mg/g) it ranged from 0.3 mg/g to 0.71 mg/g being the highest with compost 3 % treatment and the lowest with application of $CdCl_2$ 0.1%, it worthy to mention that use of $CdCl_2$ 0.5% completely prevented maize plants to grow. The treatment of $CdCl_2$ 0.1% + compost 1% increased P- content in comparison with treatment of $CdCl_2$ 0.1% as such, the percentage of increase reached 20. Increasing compost dose to 3% in the presence of $CdCl_2$ 0.1% did additional increase reached 43.3%.

Table 2: Influence of $CdCl_2$ in presence or absence of compost on dry weight and macro-nutrients content of maize plants.

Treatments \ Parameters	Dry weight (g/plant)	N-content (mg/g)	P-content (mg/g)	K-content (mg/g)
Control	7.33	2.60	0.48	5.75
Cadmium 0.1%	0.66	1.50	0.30	5.18
Cadmium 0.5%	0.00	0.00	0.00	0.00
Compost 1%	9.33	3.30	0.62	7.04
Compost 3%	11.00	4.40	0.71	9.61
Cadmium 0.1% + Compost 1%	2.00	2.10	0.36	6.00
Cadmium 0.1% + Compost 3%	3.33	2.80	0.43	6.75
Cadmium 0.5% + Compost 1%	0.00	0.00	0.00	0.00
Cadmium 0.5% + Compost 3%	0.00	0.00	0.00	0.00
LSD (0.05)	1.83	0.025	0.008	0.027

Potassium content of maize plants ranged from 5.18 to 9.61 mg/g dry weight; the lowest value was recorded with $CdCl_2$ 0.1% treatment while the highest value was found with the treatment of compost 3%.

The synergistic influence of both bio-agent *B. cereus* and compost on maize plants grown under tow levels of $CdCl_2$ (0.1% and 0.5%) was presented in Table (3). Concerning maize plants dry weight, the control treatment (without $CdCl_2$ stress) produced 7.33 g/plant while the plants treated with 0.1 % $CdCl_2$ produced dry weight reached 0.66 g/plant. Again, maize plants treated with 0.5% $CdCl_2$ completely failed to grow and produced 0 g/plant dry weight. Biofertilization of maize plants with *B.*

cereus did increase in dry weight over the control reached 18.15%. Application of 1% compost significantly augmented maize plants dry weight over the control treatment reached 27.29%; furthermore, additional increase in maize dry weight was obtained due to increasing the compost dose to 3% that reached 50.09% over the control treatment.

Table 3: The associative effect of *B. cereus* and compost in presence or absence of $CdCl_2$ on dry weight and macro-nutrients content of maize plants.

Treatments \ Parameters	Dry weight (g/plant)	N-content (mg/g)	P-content (mg/g)	K-content (mg/g)
Control	7.33	2.60	0.48	5.75
Cadmium 0.1%	0.66	1.50	0.30	5.18
Cadmium 0.5%	0.00	0.00	0.00	0.00
Bacillus cereus	8.66	3.10	0.59	6.90
Compost 1%	9.33	3.30	0.62	7.04
Compost 3%	11.00	4.40	0.71	9.61
Cadmium 0.1% + *B. cereus* + Compost 1%	2.33	2.50	0.40	6.28
Cadmium 0.1% + *B. cereus* + Compost 3%	4.00	2.90	0.47	7.64
Cadmium 0.5% + *B. cereus* + Compost 1%	0.00	0.00	0.00	0.00
Cadmium 0.5% + *B. cereus* + Compost 3%	0.00	0.00	0.00	0.00
LSD (0.05)	1.83	0.025	0.008	0.027

Biofertilization of maize plants with *B. cereus* in the presence of both compost at 1% and $CdCl_2$ at 0.1% produced dry weight reached 2.33 g/plant; the percentage of increase over the treatment that received0.1% $CdCl_2$ as such reached 253.03. In addition, increasing the compost rate to 3% (0.1% $CdCl_2$ + *B. cereus* + compost 3%) raised the increasing percent to 556.06.

For maize plants nitrogen content (mg/g), Table (3) also indicates that addition of biofertilizer *B. cereus* and compost at the rate of 1% to the treatment of $CdCl_2$ 0.1% significantly increased N-content, the increase reached 66.67% in comparison with $CdCl_2$ 0.1% treatment as such. Furthermore, additional increase in N-content of maize plants when the compost dose of 1% was replaced by 3% at the same previous treatment; the percentage of increase reached 93.33. As for P-content of maize plants, it ranged from 0.30 mg/g to 0.71 mg/g being the lowest due to the application of 0.1% $CdCl_2$ and the highest owing to the treatment of 3% compost. Application of both *B. cereus* and compost at the rate of 1% to maize plants treated with 0.1% $CdCl_2$ improved significantly P-content where the increase reached 33.33%. Additional significant increase was recorded when the compost dose was elevated to 3% where the increase reached 56.67% in comparison with application of $CdCl_2$ 0.1% as such. Regarding maize plants K- content, the same previous trend of both nitrogen and phosphorus was observed. Potassium content of maize plants ranged from 5.18 mg/g to 9.61 mg/g being the lowest with the treatment of $CdCl_2$ 0.1% and the highest due to the treatment of $CdCl_2$ 0.1% + *B. cereus* + compost 3%. Moreover, utilization of *B. cereus* and compost 1% together with $CdCl_2$ 0.1% lowered the toxic effect of $CdCl_2$ on maize plants where this treatment significantly increased K- content with 21.24%. Further, increasing the compost dose to 3% instead of 1% did more increase in K- content of maize plants to reach 47.49%.

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