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COMBINED EFFECTS OF NICKEL AND ARSENIC ON GROWTH AND MINERAL NUTRIENTS ACCUMULATION IN KALMI (*Ipomoea aquatica*), RED AMARANTH (*Amaranthus* sp.) AND SPINACH (*Spinacia oleracea* L.) AND NUTRIENT RELEASE PATTERN IN SOIL AT DIFFERENT DAYS OF INCUBATION

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Abstract

Arsenic (As) is a broadly distributed toxic metalloid that accumulates in the environment through natural and anthropogenic sources. Numerous techniques were evolved for arsenic remediation from water including adsorption, flocculation, ion exchange and reverse osmosis. Due to the excessive affinity between iron and inorganic arsenic species, iron-based adsorption is an emerging technique for the remediation of arsenic-contaminated water and soil. Whereas iron (Fe) and nickel (Ni) belong to the same chemical group (VIIIB) in the periodic table, it is expected to get similar interaction of Ni with As. An *in vitro* incubation study and pot experiment were conducted to evaluate the effect of Nickel on mineral release patterns in soil and growth yield of kalmi (*Ipomoea aquatica*), red amaranth (*Amaranthus* sp.) and Spinach (*Spinacia oleracea* L.). Nickel was applied as different doses of Nickel of 10, 40 and 160 mg/kg respectively where As was applied with irrigation water at the rates of 1 mg/L and incubated at field moisture condition for 10, 20 and 30 days individually in different pots. Total organic carbon (C), total nitrogen (N), phosphorus (P), sulfur (S), potassium (K) and 0.1N HCl extractable arsenic (As), iron (Fe) contents were determined at 10, 20 and 30 days of incubation. The pot experiment was carried out in triplicates for 45 days till the plants were grown to maturity. The growth performance of plants and the remedial effect of Ni on As toxicity in soil and plant was examined. Nickel showed a significant impact on fresh and dry yield of three plants and an antagonistic relationship between Ni and As was also observed i.e., Ni in soil was found to lessen the availability of As in soil likewise its accumulation in plants.

Keywords: Arsenic; Nickel; Biomass; Bioavailability; Incubation.

INTRODUCTION

Arsenic pollution is a recent threat to our environment predominantly derived from natural sources that occurs in groundwater in many countries, affecting the health of millions of people (Duxbury *et al*. 2003). Groundwater arsenic contamination in Bangladesh is reported to be the biggest arsenic calamity in the world in terms of the affected population (Talukder *et al*. 1998) which was first detected in 1993 (Khan *et al*. 1997). This toxic metalloid is found in soils and water existing in different chemical forms as a component of more than 245 minerals (Azcue and Nriagu 1994). This ubiquitous element can release into the hydrosphere by various means of natural processes such as weathering (dissolution of minerals), microbial activity, and complexation with natural organic compounds (Fang *et al.* 2018). Moreover, Arsenic may accumulate in soil and surface water from myriad anthropogenic activities, including industrial mining and metallurgical industries, combustion of fossil fuels (e.g., coal with high arsenic content), use of arsenical pesticides, herbicides, and crop desiccants (Hering *et al*. 2017). Crops are irrigated with As-contaminated water, possess a risk of arsenic accumulation in soil, and ultimately end up in the food chain through plant uptake and animal consumption (Huq *et al*. 2001). It may adversely affect crop quality and contaminate the soil environment. Normal levels of As in soil usually range from 4-8 mg/kg, where it may exceed 58 mg/kg when As-contaminated water is used as agricultural water (Huq and Naidu 2003).

Arsenic can exist in a range of oxidation states from -3 to +5 (Siddiqui and Chaudhry 2017), though it is most commonly found as As (III) or As (V) derivatives, depending on pH and redox conditions (Smedley and Kinniburgh 2002, Lorenzen *et al*. 1995). It is known that As (III) is more toxic (Ferguson and Gavis 1972) and more mobile than As (V) (Amin *et al*. 2006). It is difficult to remove arsenic from arsenic-contaminated soil. Arsenic has a high adsorption affinity with iron oxide and both As (III) and As (V) are strongly adsorbed and occluded by the mixed Fe (III) oxide products (Gupta *et al*. 2012). So, one of the common techniques to remove arsenic from soil is co-precipitation with Fe-bearing compounds such as goethite (α-FeOOH), iron sulfate (with lime), iron grit, etc. (Hartley *et al*. 2004). Iron and nickel belong to the same chemical group (VIIIB) in the periodic table and also share similar physical and chemical characteristics. It is expected to get similar interaction of Ni with As.

Nickel (Ni) is an indispensable component of the urease (Dixon *et al*. 1975) and hydrogenase enzyme (Evans *et al*. 1987) which plays a key role in nitrogen metabolism-was the rearmost nutrient to be recognized as an essential element for plants. Even though plants usually have a low demand for this micronutrient (Seregin and Kozhevnikova 2006), numerous reports have shown that Ni deficiency has a wide range of effects on plant growth and metabolism including N metabolism and Fe uptake (Brown *et al*. 1987). Preliminary investigations additionally imply that Ni may have a role in the synthesis of phytoalexin and plant disease resistance (Graham *et al*. 1985). Contrastingly, excess concentration of Ni in plants causes chlorosis and necrosis, due to disruption of Fe uptake and metabolism (De Kock 1956). Elevated concentration of Ni can inhibit cell division at root meristems in non-tolerant plants (Robertson and Meakin 1980) and decrease plant growth (Foy *et al*. 1978). On the other hand, kalmi (*Ipomoea aquatica*), red Amaranth (*Amaranthus* sp.) and spinach (*Spinacia oleracea* L.) are three popular vegetable crops that are widely cultivated in Bangladesh (BBS 2021). These vegetables also provide us a considerable amount of essential nutrients, minerals and vitamins. These plants have the ability of high biomass production and have translocation capacity of As and Ni from soil to pants. To assess the effects of Ni on plants in relation to As, these plants were chosen for pot experiments.

The present experiment aims to-(a) determine the combined effect of Ni and As on soil and plant health, (b) assess the impact of Ni on several nutrient release patterns in soil and (c) evaluate the impact of Ni to reduce the availability of As in soil and plants.

MATERIAL AND METHODS

Collection of soil samples for pot experiments

The bulk of soil samples was collected by composite soil sampling method from Manikgonj district (23˚52'60"N and 90˚02'12"E), representing 0-15 cm depth from the surface subsequently dried and grounded. The collected soils were Young Brahmaputra Floodplain belonging to the Melandaha series. Then, a part of soil samples was screened to pass through a 2 mm sieve and used for various physical analyses and also for the determination of organic carbon content of soil. A portion of soil samples (2 mm sieved) was further grounded and screened to pass through a 0.5 mm sieve and prepared for analyzing various chemical and physico-chemical parameters of the soil. The bulk soil sample for pot experiment was air-dried, crushed, cleansed and screened through a 5 mm sieve and used for the pot experiment.

Net-house experiment

For the pot culture experiment, three leafy-vegetable crops kalmi (*Ipomoea aquatica*), red amaranth (*Amaranthus* sp.) and spinach (*Spinacia oleracea* L.) were selected. A total of 36 pots with 1 kg soil each were prepared, where for each test plant species 3 pots were control and 9 pots were supplied with 3 different treatments of nickel (10, 40 and 160 mg/kg) and arsenic (1 mg/L). Nitrogen (N), phosphorous (P), potassium (K) and sulfur (S) fertilizers were supplied to ensure the optimum growth of the plants as recommended by BARC (2015). The source of Ni was $NiCl_2$ and different doses of Ni were made from a 1000 mg/kg stock solution of Ni. Arsenic dose of 1 mg/L strength was applied with 100 ml of irrigation water every day after the 7 days of seed plantation. The solution was made in combination of 80% Na-meta arsenite ($NaAsO_2$) and 20% Na-arsenate ($Na_2HAsO_4.H_2O$). The control treatments did not receive either Ni or As.

(a) (b) (c)

Fig. 1. Plot culture before hervasting: a. Kalmi plants; b. Red amaranth plants; and c. Spinach plants.

Collection of samples after pot experiment

The plants were allowed to grow for 45 days and were harvested by uprooting them carefully from the pots. Then, after washing with deionized distilled water and weighing for fresh weight, the samples were cut, oven-dried and weighed for dry weight. The oven-dried plant samples were then grounded with an electrical grinder, screened through a 0.2 mm sieve and digested with concentrated nitric acid for further laboratory analysis. Meanwhile, after harvesting the plant soil samples were collected from each pot; were then air-dried, grounded and passed through a 0.5 mm sieve for subsequent analysis.

Experimental setup for in-vitro incubation study

For the incubation study, 39 pots were prepared with 150 g air-dried 5 mm sieved soil sample and 3 treatments of Ni (10, 40 and 160 mg/kg) and As (1 mg/kg). Either Ni or As was not added to the control treatments. Then the soils were incubated at field moisture condition for 0, 10, 20 and 30 days, respectively. Thereafter, the soil samples from each pot were collected randomly for further analysis.

Analyses of soil and plant samples

Various physical properties- Textural classes, moisture content and the particle size analysis and physico-chemical properties- pH, organic carbon, phosphorous, potassium and nitrogen content of the soil samples were determined as described in Huq and Alam (2005) and USDA (1951) which are shown in Table 1.

The As content of the soil (both initial and after harvest) and plant samples were determined from the extract by Hydride Generation Atomic Absorption Spectrophotometry (HGAAS) with the help of 50% KI and 10% Urea in acid medium using the method of Voth-Beach and Shrader (1986). The total Fe and Ni content of the soil (both initial and after harvest) and plant samples were determined by a Varian Atomic Absorption Spectrometer (AAS) model 220 after extraction of the samples with concentrated nitric acid (HNO_3).

Table 1. **Physico-chemical properties of the soil used (0-15 cm depth).**

Soil Properties	Values	Soil Properties	Values
pH	6.61	Total Nitrogen (%)	0.097
Sand (%)	13.9	Available Phosphorous (ppm)	3.23
Silt (%)	74.1	Available Potassium (me/100g)	0.11
Clay (%)	12.0	Available Sulfur (ppm)	10.17
Textural class	Silt Loam	Total Arsenic (ppm)	3.21
Moisture Content (%)	21.54	Iron (%)	1.68
Organic Carbon (%)	1.3	Nickel (ppm)	0.728

Statistical analysis

All data in the study were calculated and graphically evaluated using Microsoft Excel. The results of the experiment were statistically analyzed by Minitab version 17.

RESULTS AND DISCUSSION

To know the initial nutrient status (N, P, K, S, Fe) and heavy metals (As, Ni) content of the soil, some common physical, chemical and physico-chemical properties were determined before the setup of the experiment which are shown in Table 1. The pH of the soil was 6.61 and the texture was silty loam. The water-extractable As content was found below the detection level (0.02 mg/L). Different doses of Ni have different impacts on fresh and dry matter yield of Kalmi, Red Amaranth and Spinach plants as observed in Table 2 where values are presented as the averages of three individual replications.

Table 2. **Fresh and dry matter production of Kalmi (g/100 plants), Red amaranth (g/100 plants) and Spinach (g/100 plants) with different doses of Ni.**

Treatment	Kalmi		Red amaranth		Spinach	
	Fresh Weight	Dry weight	Fresh Weight	Dry weight	Fresh Weight	Dry weight
Control (-Ni & -As)	61.02	6.02	27.57	2.42	22.78	1.54
$Ni_{10}As_1$	121.11	10.93	34.61	3.22	32.18	1.91
$Ni_{40}As_1$	99.26	9.97	11.21	0.99	17.46	1.11
$Ni_{160}As_1$	123.09	11.57	30.83	2.48	20.83	1.24
F value	7.726^{**}	4.156^{*}	4.657^{*}	2.532^{ns}	4.156^{*}	3.971^{ns}

** = significant at 1% level, * = significant at 5% level, ns = not significant.

Table 2 shows that $Ni_{10}As_1$ treatment produces the highest amount of fresh matter and dry matter for both Red Amaranth and Spinach. Though, this treatment produces a higher amount of fresh and dry matter of kalmi, but $Ni_{160}As_1$ dose produces the highest amount of fresh and dry matter of kalmi. On the other hand, $Ni_{40}As_1$ treatment produces the minimum fresh and dry matter yield of red Amaranth, Spinach and control treatment produces the minimum fresh and dry matter of kalmi. The table also shows that, control treatment (without Ni and As dose) produces a low yield of fresh and dry matter. It can be concluded from Table 2, a low level of Ni dose ($Ni_{10}As_1$) produces the best yield as a higher amount of Ni can decrease plant growth (Foy *et al*. 1978).

The concentration and uptake of As, Fe and P in kalmi, red amaranth and spinach were analyzed to evaluate the effect of Ni on the accumulation in plants over the control soil (Fig. 2 to 4). It was observed from Fig. 2b that, initially $Ni_{10}As_1$ treatment showed the maximum As accumulation in plants and then again with the increased rate of Ni dose, As accumulation was found to be decreased, though red amaranth showed a slight increase in As uptake at 160 mg/kg Ni dose. But there is a different trend observed for Fe and P accumulation (Fig. 3b) and Fig. 4b. Fig. 3a showed that there was a gradual increase in Fe concentration in all three plants with the increased level of Ni. There may be uninterrupted nutrient uptake by plants. So, the concentration of Fe increased and showed a synergistic effect on each other. $Ni_{160}As_1$ treatment showed the maximum accumulation of Fe in plants (Fig. 3b).

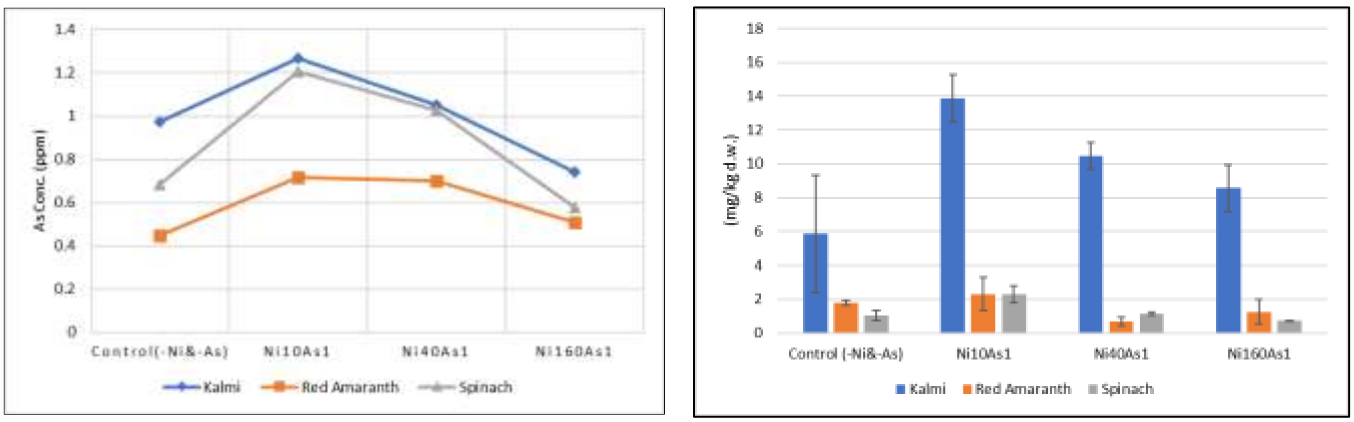

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Fig. 2a. As concentration (ppm) in plants, b. As uptake by plants (mg/kg d.w.) with different doses of Ni. Error bars represent the standard deviations (SDs).

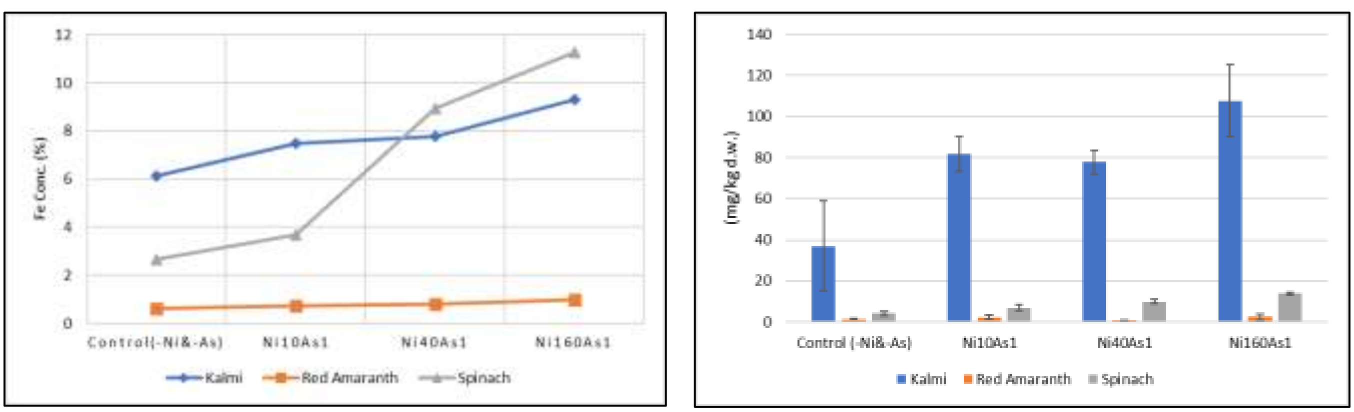

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Fig. 3a. Fe concentration (%) in plants; b. Fe uptake by plants (mg/kg d.w.) with different doses of Ni. Error bars represent the standard deviations (SDs).

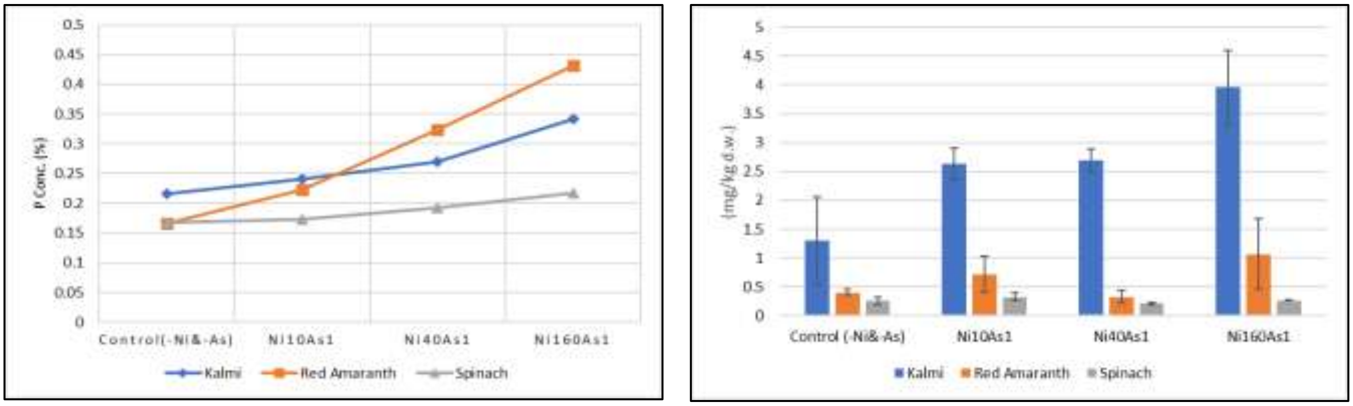

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Fig. 4a. P concentration (%) in plants; b. P uptake by plants (mg/kg d.w.) with different doses of Ni. Error bars represent the standard deviations (SDs).

The concentration of P in kalmi, red amaranth and spinach is presented in Fig. 4b, where we observed that there is a gradual increase in P concentration in plants with the increase of Ni treatment. Though the doses were four times more than each other, the increase in P concentration is quite static. Phosphorus uptake in plants was initially increased with Ni and then slight decrease and again increased with the increasing dose of Ni. According to Atkinson *et al*. (2010), the availability and subsequently the adsorption of P are highly pH dependent. Increases in soil pH are likely to influence P availability, with available forms most common between 4-8.5 pH levels. There is a significant effect of treatments on P concentration and uptake at 5% level.

With the application of Ni dose, there is a variation in pH observed (Fig. 5). It is found that, the pH of Ni-treated soil initially slightly increased at 10 days of incubation followed by a decline at 20 days of incubation and again the pH of the soil increased with further incubation time. The initial decrease and then increase in soil pH could possibly be due to the chemistry of submerged soil. Motomura (1962) and Ponnamperuma (1965) reported that, when an aerobic soil is submerged, its pH decreases to a minimum during the first few days, and then increases asymptotically to a fairly stable value of 6.7-7.2 a few weeks later. The result also suggests that, a higher dose of Ni slightly increases the pH of the soil.

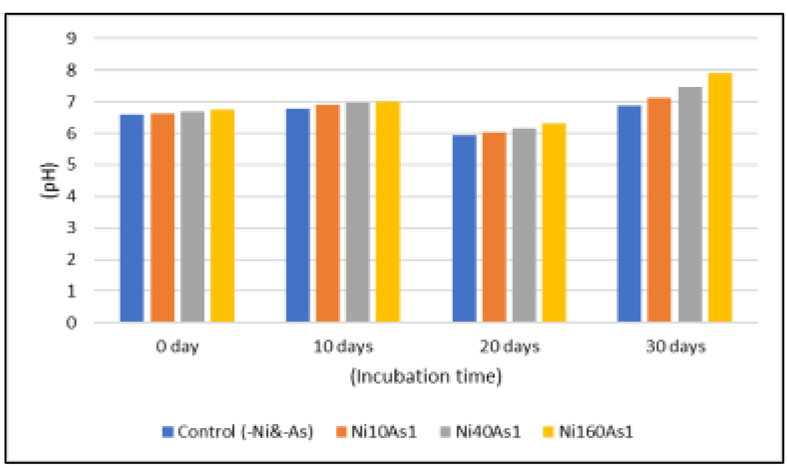
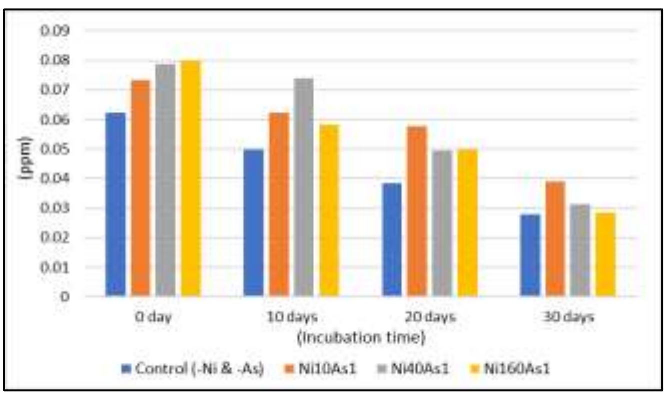


Fig. 5. Change in soil pH over a 30 days incubation period with different doses of Ni.

In this experiment it was found that all the treatments had no significant impact on total nitrogen (N), available nitrogen (N), sulfur (S) and potassium (K) (data not shown) where the availability of S and N in the treated soils showed inconsistent responses. The available K content was almost static as the initial background level of the soil. The effects of the application of all treatments of Ni dose on available As, Fe and P in soil were found to be significant and are presented in Fig. 6. Nickel treatments showed an antagonistic relationship with As (Fig. 6a). $Ni_{10}As_1$ treatment showed the maximum As availability in soil in the initial days of incubation, but with the increased dose of Ni and incubation time, there is a decrease in As availability in soil was observed. Statistical analysis shows both Ni doses and incubation time have a significant impact on As availability in soil (P<0.01). Fig. 6b. suggests a synergistic effect between Ni and Fe. It was also observed from the figure that, in control soil, the availability of Fe was almost constant with incubation time. The increase of Fe availability in soil with the application of Ni doses was highly significant (P<0.001). After the application of Ni, it has shown a peak at 20 days of incubation that was, with time the availability of Fe increases. Fig. 6c showed a significant effect of Ni doses on P availability in soil and the impact of incubation time on available P content was also found to be significant at 1% level. It was observed from Fig. 6 c that, in control soil, the availability of P initially increased, but at 20 days it decreased and again increased at 30 days which is similar to the findings of Kaloi *et al*. (2011); the highest availability of P observed at 20 days of incubation with the dose of $Ni_{40}As_1$. At 30 days of incubation, P availability decreased with $Ni_{160}As_1$ dose which might show toxicity due to over-dose of Ni application.



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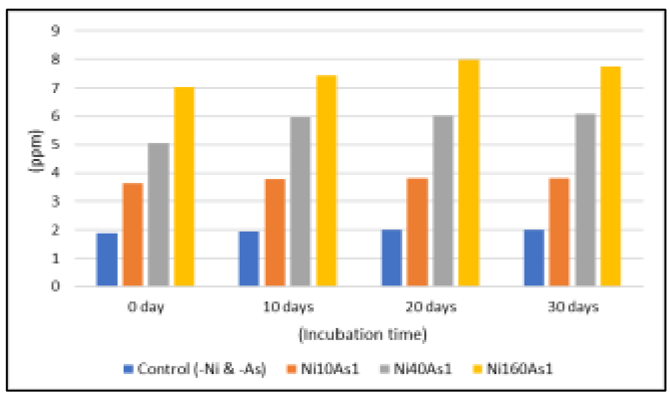

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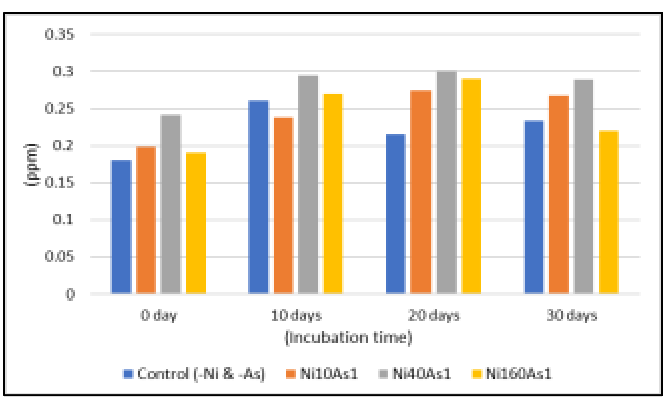

c

Fig. 6. Effects of Ni doses on: a. Arsenic concentration; b. Available Fe; c. Available P in soil at different incubation times.

It can be concluded from the present study that increasing doses of Ni can suppress the As availability in soil and furthermore reduce the plant As concentration. In addition, combined doses of Ni and As showed a positive impact on available Fe and P. So, within the critical limit value of Ni for plants, Ni increases the growth yield of plants, reduces As concentration and affects mineral release patterns in soil.

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