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Persistence of Pathogens in Heavy Metals Contaminated Environment

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Abstract: A prospective study has been carried out to ascertain the persistence of pathogens in heavy metals contaminated environment at SITE and Hub river industrial area Karachi. The vegetable (spinach) was selected to analyze heavy metals contents like Co, Hg, Ni, Pb, Fe, Na and K. Spinach growing in this contaminated water contain a great number of pathogens like *E. coli* (fecal Coliforms), Staphylococci, Bacillus, Aeromonas, Salmonella, Shigella and Vibrios. More over most of these organisms were found to be resistant against a variety of antibiotics. Microbes were isolated by standard methods by utilizing different selective medias for their growth and their sensitivities were determined by disc diffusion method. Metals were analyzed by atomic absorption spectrophotometer.

Key words: Spinach, heavy metals, pathogenic microorganisms, antibiotic resistant

INTRODUCTION

Pollution is a global problem and Pakistan being an under developed country faces a tremendous threat of pollution. The environment specially the soil was increasingly polluted with heavy metals and a number of potentially toxic elements mainly in urban agglomeration that found to promote the growth of multi drug resistant Pathogenic microorganisms.

Accumulation of heavy metals^[1] from the sewage sludge's can effect on crops biomass and liming^[2] is often recommended to minimize the plant uptake of potentially toxic elements from sludges-amended soils. Living thing takes up these toxic metals through food, water and air. The field experiment determines the extent of Cd, Pb and Zn uptake by vegetables from fields formerly irrigated with municipal waste water from 10³ years periods. Thus, compared to vegetables grown on neighboring agricultural field, leaf vegetables (e.g. cabbage, lettuce, spinach^[3]). Up take of Cd and Zn was considerable (2.4-13.5 and 21.2-1048 $\mu\text{g g}^{-1}$ for Cd and Zn, respectively) yet Pb accumulation was low and compare able (0.3-3.3 $\mu\text{g g}^{-1}$). Root vegetables (potato, onion), Cd and Zn level were generally lower than those in leaf vegetables^[4].

Plants are more sensitive to pollution than animals or man. Trace elements play a very important role in chemical, biological, biochemical and enzymatic reaction in the living cells of plants, animals and human bodies^[5].

The role of trace elements is significant for body. Their deficiency causes diseases, where as in excess, these trace elements cause toxicity to human life. Vegetables grown in industrials waste water, contaminated by these elements and pathogens cause several diseases affecting hundred million of peoples every year all over the world. Isolation of staphylococcus has been reported^[6] in ready to eat vegetable salad. Since seeds contain^[7] sufficient nutrients to support microbial growth, nutrient leading could faster growth both in the soak water and on the seed surface vegetables and fruits get contaminated with pathogenic microorganism.

Ni, Co, Zn and Mn are accumulated by the fast and unspecific CorA magnesium uptake system in Gram-negative bacteria^[8], archaea and baker's yeast^[9]. Arsenate is transported by the fast Pit-phosphate system and chromate by the fast sulfate uptake system^[10]. In addition, there are inducible P-type ATPases for magnesium uptake, ABC transporters for Mn, Zn and Ni, slow and specific chemosmotic transporters of the Hox N-family for Ni and Co and ABC transporters as well for sulfate and phosphate in bacteria^[11].

Present investigation was carried out to detect the heavy metals accumulation from soil in vegetables growing in municipal and industrial wastewater of SITE and Hub river industrial area Karachi and to study the microbial activity of extract in different solvents with different pathogens. Results have been interpreted in terms of microbial activity of drug resistance pathogens.

MATERIALS AND METHODS

All chemicals used were purchased from Merck/Oxoid Analanar grade. Fresh spinach samples were collected from Sindh Industrial Trading Estate Karachi and Hub river SITE Spinach samples were washed thoroughly with distilled water and air-dried.

Five grams of dried sample were wet ashed with 40 mL (1:1) $\text{HNO}_3\text{-HClO}_4$ and heated to near dryness in a platinum dish, few drops of hydrochloric acid were added and heating was continued to dryness, the residue was treated with 10 mL concentrated HCl and boil for 30 min then 20 mL distilled water was added and solution was heated for further 15 min and made up to 50 mL^[12].

Heavy metals analysis were carried out by Hitachi Z-8000 atomic Absorption spectrophotometer meter with Zeeman correction using air acetylene flame with standard addition methods.

Microbiological analysis: Extracts were prepared in three different solvents, like Hexane Methanol and Water and subjected for Microbiological analysis like Streak plate method/SPC (standard plate count) and Coliforms detection, different dilutions of extract were prepared and incubated with pathogenic microorganisms at 37°C, different selective medias were used for the isolation of certain organisms like KG agar was used for *B. cereus*^[13]. The isolates were identified and confirmed by biochemical tests. Fresh leaves of spinach were soaked in sterile^[14] saline for over night and organisms were isolated by using different selective medias. Antibiotic sensitivities were also determined. All procedures were performed by methods approved by ASM (American Society of Microbiology) manual.

RESULTS AND DISCUSSION

Karachi being an industrial city comprises a net work of many small and big industries. The industrial areas are not far away from the residential areas and because of industrial waste other hazardous material, heavy metals are also present in its air, water and soil sediments which not only effect the nutritive values of fruits and vegetables but also have deleterious effect on human being using these in turn. Heavy metals have great significance due to their tendency to accumulate in the vital human organs over prolonged period of time. Plants are more sensitive to pollution than animals or more. Human using these toxic vegetables gets dangerous effect on their body because vegetables grown in this polluted area carry germs and high percentages of heavy metals.

Table 1: Concentration of heavy metals (ppm) in spinach (Gutter baghicha and Hub river SITE)

Metals	Fresh weight of spinach (g)	Concentration of metals in dilute sample (Gutter baghicha SITE)	Concentration of metals in dilute sample (Hub river SITE)
Co	5	1.61	1.61
Hg	5	57.20	87.80
Ni	5	1.14	0.87
Pb	5	62.30	80.10
Fe	5	60.20	67.20
K	5	39.45	41.34
Na	5	33.33	34.50

Table 2: Growth pattern of different microorganism grown in spinach extract

Organism	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	C ₂	C ₁
<i>E. coli</i> [2949]	++++	++++	++	++	++	++++	++++
<i>E. coli</i> [2932]	++	++	++	++	++	++++	++++
<i>Proteus</i>	++++	++++	+++	+++	++	++++	++++
<i>Klebsiella</i> 2955	++++	++++	++++	++++	++++	++++	++++
<i>Klebsiella</i> [2927]	+++	+++	+++	+++	++	++++	++++
<i>Pseudomonas aeruginosa</i>	+++	+++	+++	++	+	++++	++
<i>E. coli</i> UT	++++	++++	+++	++	+	++++	++
<i>Staph aureus</i>	++++	++++	+++	++	+	++++	++
<i>S. typhi</i>	++++	++++	+++	++	+	++++	+++
<i>Klebsiella</i>	++++	++++	+++	++	++	+++	+

C₁: spinach extract, C₂: spinach+broth

Table 3: Organisms-isolated *Shigella* sp., *Salmonella* sp., *P. mirabilis*, *K. pneumoniae* and *Pseudomonas* sp. *E. coli*

Antibiotic (μg) resistance pattern of <i>K. pneumoniae</i> isolates	Resistance (%)
Ceftazidime(30)	80
Cefotaxime (30)	75
Ceftriaxone (30)	80
Amikacin (30)	70
Ampicillin (10)	75
Co-trimoxazole (25)	50
Gentamicin (10)	60
Cefuroxime (30)	90
Cephaloridine (30)	80
Tetracycline (30)	60

Spinach, which is usually used by the peoples of city grown in sewage of untreated liquor of industries and other, polluted materials.

Spinach grown in the waste water region of SITE area and Hub river side was reanalyzed for Co, Hg, Ni, Pb, Fe, K and Na. It has been found that spinach is not useful for the health of human beings because it contain high percentages of heavy metals which support the growth of micro-organisms even after heating it supports the growth of pathogens, because of these reasons food born illnesses are going to rise day by day in third world countries. The results have been shown in Table 1.

Antimicrobial activity: Spinach extract in different solvents like n-hexane, methanol and water when subjected to check the microbial activity, surprisingly it gave unexpected results i.e it enhanced the growth of all pathogens (Fig. 1 and Table 2) instead of inhibiting their



Fig. 1: Growth of organisms in different dilutions of extract



Fig. 4: Positive Coli forms test in methanolic extract



Fig. 2: Growth of organisms in Pb precipitates



Fig. 3: Positive coliforms test results of spinach extract

growth although the extracts were prepared in methanol and n-hexane which is famous for its antibacterial activity or antimicrobiological activity.

It was also found that when water extract of spinach was treated with $K_2Cr_2O_7$. It gave precipitates when these precipitates were tested for antimicrobial activity they were found to enhance the growth of organisms (Fig. 2).

In case of n-hexane and methanols, respectively high concentration of heavy metals may be responsible for the growth of organisms, microbiological growth should be



Fig. 5: Growth of organisms on different media, isolated from spinach

diminished as methanol and n-hexane are antibacterial even then microbial growth enhancement was observed (Fig. 3 and 4).

Fresh leaves of spinach were soaked in sterile saline for over night and organisms were isolated by using different selective medias (Fig. 5). Antibiotic sensitivities were also determined, isolated organisms were found to be resistant against a variety of antibiotics (Table 3).

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