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Individual and combined toxic effect of nickel and chromium on biochemical constituents in *E. coli* using FTIR spectroscopy and Principle component analysis

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ABSTRACT

Ni and Cr are ubiquitous pollutants in the aquatic environments. These heavy metals elicit toxicities to aquatic organisms including microbes. In this study, interaction of the two heavy metals on the toxicity in *Escherichia coli* (*E. coli*) was studied using FTIR spectroscopy. The binding of Ni(II) to *E. coli* was stronger than that for Cr(VI). Cr exhibited antagonistic effects in the presence of Ni in *E. coli*. FTIR analysis showed a decrease in lipid content in the presence of Ni and not for Cr. Further, a decrease in band area was observed in the region of 3000–2800 cm^{-1} and at ~1455 cm^{-1} due to a decrease in fatty acids and lipid molecules. The band area ratio of lipid was used to monitor the changes in fatty acids due to metal toxicity. Principle component method helps to discriminates the results between control and metal toxicities in *E. coli* from the FTIR data. The study shows the importance of metal interaction and its toxicity on *E. coli*.

1. Introduction

Heavy metals constitute a serious form of pollution since they do not degrade as organic pollutants. Many metallic ions form stable complexes or chelates and they can act like cumulative poisons, which results in a serious threat to the aquatic system. Ni is widely distributed in nature and the main use of Ni is in the steel industry, such as alloys, chemical catalysts in electroplating metals, production of ceramics, pigments, NiCd batteries, and coins (Schroeder, 1973). Its deficiency may affect the carbohydrates, lipids and iron metabolism in biological organism (Nielsen and Ollerich, 1974; Kirshgessener and Schnegg, 1976). Nickel salt has been shown to react with DNA and alter the constituents and function of nucleic acids in biological system (Khangarot and Ray, 1990; Hui and Sunderman, 1980). Cr is a relatively scarce metal and it arises from industrial effluents derived from the production corrosion inhibitors, pigments, leather tanning, electroplating, metal finishing and chromate preparation (Srivastava et al., 1979; Galvin, 1996). Chromium particularly in its hexavalent from is a well known highly toxic metal, considered as a priority pollutant (Zouboulis et al., 1995; Sharma and Forster, 1993). Recently it has been acknowledged that multiple chemicals at substantially lower concentration than the established water quality standards can cause toxicity when acting jointly. Hence it is essential to develop techniques to understand, analyze and predict toxic effects caused by multiple chemicals acting jointly. In analyzing toxic effects in binary mixtures, Plackett and Hewlett (1952) identified modes of primary action of the two chemicals whether one chemical does or does not influence others. If the response is caused by both chemicals, than the two are said to have joint effects. This joint effect can be classified into adding more that additive or less than additive.

Escherichia coli are Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* which is harmless in most cases, but some strains can cause serious food poisoning in their hosts. This bacterium can be grown and cultured easily and inexpensively in a laboratory setting. Quintelas et al. (2009) studied the absorption of heavy metal Ni, Cr, Fe and Cd by *E. coli* and its biochemical changes using AAS and FITR. FTIR spectroscopy has been successfully applied for detection, discrimination, classification of bacteria and other microorganism. The wave number positions of absorbance peaks, peak intensities and peak widths are useful for functional group, cell component, and sample identification. It has proved to be powerful and successful analytical technique that provides qualitative and quantitative information.

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The interpretation of spectra and peak assignments are key steps in the FTIR analysis of any biological sample. The most functional group vibrations in the case of microorganisms are proteins, fatty acids, nucleic acid and carbohydrates (Davis and Mauer, 2010). It allows rapid, accurate, reproducible, cost effective in the determination of molecular alterations in membranes, cellular and tissue levels (Barth and Haris, 2009; Cakmak et al., 2011; Karthikeyan and Mani, 2014). It has high sensitivity in detecting small changes in the functional group of biological molecules and gives information about membrane fluidity, lipid order, and the contents of saturated lipids, unsaturated lipids, cholesterol ester, Proteins, RNA, DNA, glycogen and nucleic acid conformation in a single tissue sample (Severcan and Haris, 2012; Siebert, 1995). The use of chemometric tools in the data analysis together with recent advances in computer technology which simplify complex mathematical calculation leads to the development of multivariate data analysis as a powerful tool in the evaluation of biochemical composition. The Principle Component Analysis is used for data reduction to identify a small number of factors in a larger number of variables. It permits a primary evaluation of in between category similarity and is very useful for visual inspection of complex data matrices (Vannajan et al., 2009). This paper describes the work carried about to determine the sub lethal concentration of Ni, Cr along with its mixtures on *E. coli* and its biochemical constituents changes using FTIR and PCA method.

2. Material and methods

2.1. Test chemicals

Nickel sulphate and Potassium dichromate were purchased from Sigma–Aldrich Company, Bangalore, India and used without further purification. The purity was atleast 99.5% and 99.7% for $NiSO_4$ and $K_2Cr_2O_7$ respectively. The various concentrations of the metals were obtained from stock solution by dilution method. It was used further for toxicity study.

2.2. Test microorganism

A loopful of *E. coli* culture was inoculated in plain St Nutrient broth and incubated at 37 °C for 24 h at shaker conditions (400 × *g*). This was used for further analysis.

2.3. Minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) for *E. coli* was performed for heavy metals -Nickel sulphate ($NiSO_4$) and Potassium dichromate ($K_2Cr_2O_7$). A sterile nutrient broth containing varying concentrations (100–500 µg/L) of metal was prepared. A 24 h old culture suspension of *E. coli* was used for inoculation and the tubes were incubated at 37 °C for 24 h. All the sets were performed in triplicates. The MIC values were interpreted when the culture reaches an OD_{600} of 0.55–0.59 [CL 157 (ELICO)]. All the tests were carried out in triplicate. The MIC results showed that *E. coli* could tolerate Ni at 258 µg/L and Cr at 193 µg/L. For metal interaction studies equal concentration of Ni and Cr salt solution were used in the range of 10–150 µg/L. From the various metal concentrations, the MIC value for metal mixtures was found to be 87 µg/L for *E. coli*.

2.4. Experimental study

For the toxicity study in *E. coli*, the sub lethal concentration (⅓rd MIC) of Ni and Cr were taken from our study in St Nutrient broth. The control sample was taken without addition of any toxicant. For metal interaction study ⅓rd MIC(87 µg/L) at three different proportions of concentration of Ni and Cr (1:1, 1:2, 2:1) was taken and the sample was harvested at the end of the period. The cells were separated by centrifugation at 10,000 × *g* for 10 min and the pellet was resuspended in sterile normal saline (0.8% NaCl). The samples were sent to IIT SAIF, Bombay for FTIR analysis.

2.5. FTIR analysis

ATR-FTIR measurements, in the range of 4000–400 cm^{-1} were recorded using a micro ATR cell FTIR accessory (Burker optic, Germany). The FTIR spectrometer was fitted with N_2 cooled MCT (Mercury-cadmium-Telluride) detector. The sample of 2 ml of *E. coli* solution is used in ATR cell for spectral measurement. For each spectrum 50 interferograms were co added. The spectra were collected at a 4 cm^{-1} resolution. A total of 2 scans was taken for each spectrum, for three replicate samples. The spectra were recorded at SAIF, IIT Bombay. The collected spectra was further analysed by origin 8.0 software.

2.6. Statistical analysis

Principal component analysis was performed using the Factor reduction method. Six samples each of 3 replicates with a total of 18 samples were used to carry out the Principle component analysis in their absorbance region as 3000–2800 cm^{-1} – Fatty acids and Lipids, 1700–1500 cm^{-1} – Proteins, 1250–1080 cm^{-1} – Nucleic Acid, 1070–1020 cm^{-1} – Polysaccharides and RNA & DNA in the region of 1000–900 cm^{-1}. The input absorption values of the sample are formed as (n × k) data matrix that transforms original measurement variables into new uncorrelated variables called principal components. The PCA transforms the input into scores and loadings which are the characteristics of principal components. The two-factor loadings were plotted to collect information on the principal components responsible for variability in the FTIR data.

A one way analysis of variance was used to illustrate the significant difference between the experimental group and control. The results were expressed as mean ± standard deviation. All the statistical analysis was carried out with SPSS 16.0 software.

3. Results and discussion

3.1. Principle component analysis of biochemical compositions of E. coli due to metal toxicity

The orientation plot of the PCA is shown in Fig. 1. The plots

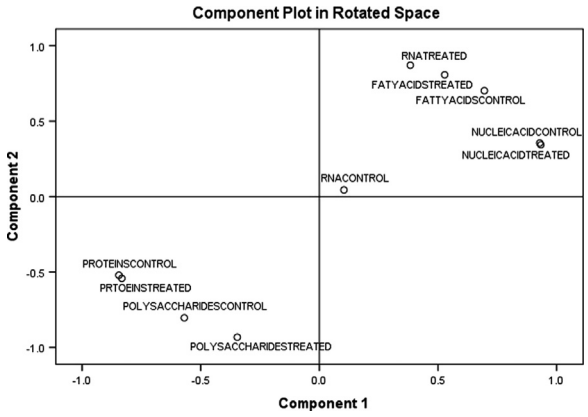

Fig. 1. PCA score plot of Biochemical constituents of *E. coli* PC1 VS PC2.